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Table of Content

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Full Length Research Paper

Etiological and predictive factors of Acute Meningitis in Mansoura fever hospital, Egypt

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Meningitis is a public health concern. It is caused by several etiologic agents that vary by age group and geographical area. This study aims to highlight the etiological and predictive factors of acute meningitis in hospitalized febrile patients in Mansoura Fever Hospital, Egypt. This study includes cases admitted with suspected meningitis. The study is conducted in the period between April 2019 and March 2020. Lumbar puncture, CSF examination and blood culture and sensitivity were done. Brain Magnetic Resonance Imaging (MRI) was performed before lumbar puncture in some patients. Detailed analysis of epidemiologic characteristics, clinical data, laboratory findings, the causative organisms and predictors of patients with Bacterial Meningitis (BM) were studied. This study included 110 patients had BM with CSF leukocytosis > 100 cells/mm3. Out of 110 CSF samples, 95 cases (86.4%) pathogens were detected by direct Gram-stained smear. Gram positive cocci were the commonest microorganism isolated. 66.4% of patients had blood culture growth of the same organism as the CSF culture. Reagent strip CSF examinations showed a positive correlation compared with laboratory tests. BM had 22.7% mortality rate. Predictive factors of poor outcomes include CSF/serum glucose ratio >0.6, CSF protein >80 mg/dl and Tonsillitis. Prognostic factors that are associated with poor outcome include old age, late presentation, delayed antibiotics treatment, neurologic complications and Glasgo Coma Scale (GCS). BM remains a leading cause of morbidity and mortality,so early diagnosis and treatment decrease both. Predictors of poor outcome of BM are CSF/serum glucose ratio >0.6, CSF protein >80 mg/dl and Tonsillitis.

Key words: Bacterial meningitis, lumbar puncture, cerebrospinal fluid (CSF) analysis.

INTRODUCTION

Acute infectious meningitis is a lethal infection of the central nervous system that leads to 422,900 deaths and

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2628,000 disable patients all over the world (Portnoy et al., 2015). Microorganisms reach the meninges either by direct spread from the ears, nasopharynx, cranial injury or congenital meningeal defect, or via bacteremia (WHO, 2017). The annual global prevalence of bacterial meningitis is over 1.2 million patients (Mount et al., 2017). Bacterial menigitis has high mortality of 50% of patients if left untreated (CDC, 2015). Furthermore, patients who were rapidly diagnosed and early treated had a mortality rate ranged from 8 to 15% within 24-48 h of onset of meningitis symptoms (CDC, 2017; Thigpen et al., 2011). In addition 10-20% of survivors may develop neurological squalea that may include permanent brain damage, cognitive dysfunction in the form of reduced processing speed that was found in one third of patients in addition to hearing loss (Rosenstein et al., 2001).

Bacterial meningitis (BM), is a serious disease that is characterized by infection and inflammation of the meninges, spinal cord and may spread to brain parenchyma, resulting in significant morbidity and mortality (Abdelkader et al., 2017). The most common infective bacteria that cause bacterial meningitis include, *Neisseria meningitis*, *Streptococcus pneumonia*, *Haemophilus influenza*, *Streptococcus Group B*, *Staphylococcus aureus*, and *Listeria monocytogenes* (Afifi et al., 2007).

Viral meningitis is generally considered a benign, self-limited disease with low mortality. Certain viruses can meningitis such as varicella zoster virus (VZV), cytomegalovirus (CMV), 6 JC virus, herpes simplex (HSV), enterovirus, Epstein- Barr virus and human herpes virus. Atypical microorganisms were also found in immunocompromised patients at increased risk of bacterial meningitis (Van Veen et al., 2017). Non-communicable diseases that are characterized by inflammation could also lead to meningitis such as malignancy, certain drugs and blood after subarachnoid hemorrhage (Jarvis et al., 2010).

Meningitis is divided clinically into acute and chronic diseases. Acute meningitis develops over hours or days, and is caused by a variety of infectious agents. Chronic meningitis has an onset that may last for weeks to months, but is generally determined when symptoms, signs, and the CSF remain abnormal for at least 4 weeks (Nath, 2016). So several infectious and non-infectious diseases characterized by chronic meningitis also exist (McGill et al., 2016). The typical clinical features of acute meningitis include clinical trial of: headache, neck stiffness and fever. Photophobia and vomiting are often present. In acute bacterial infection other constitutional symptoms may be present such as intense malaise, fever, rigors, severe headache, photophobia and vomiting. This develops within hours or minutes. The patient is irritable and often prefers to lie still. Neck stiffness and a positive Kernig's sign usually appear within hours (Sa˘glam et al., 2013). The clinical manifestations and CSF examination should determine a presumptive cause of acute meningitis within few hours. Typically, treatment must be initiated rapidly before confirming diagnosis of the actual organism is identified to decrease morbidity and mortality of this serious infection (Amarilyo et al., 2011).

MATERIALS AND METHODS

Bacterial meningitis is still a life-threatening disease that is associated with significant mortality and morbidity. Prognostic factors associated with poor outcomes were old age, neurological complications, Glasgow Coma Scale and late administration of antibiotics so early therapeutic interventions are of utmost importance to save life.

Patients

This cross-sectional study was conducted in Mansoura fever hospital, in the period between April 2019 and March 2020. Informed consent obtained from all patients and study protocol was approved by the ethical committee of Faculty of Medicine, Zagazig University.

Study population

This study included 350 patients admitted to Mansoura fever hospital is specialized 50 bed fever hospital that is located on The Nile River 250 Km east of Cairo, Egypt with suspected meningitis during the period of the study.

Inclusion criteria

Any person with sudden onset of fever and one of the following signs: neck stiffness, altered consciousness, other meningeal signs or purpura fulminans. Any child under 2 years old age with sudden onset of fever and one of the following signs: neck stiffness, or flaccid neck, bulging fontanel, convulsion, other meningeal signs or purpura fulminans.

Exclusion criteria

Cases admitted with suspicious meningitis following head trauma or neurosurgical procedure, or cerebral abscess.

Methods

All patients underwent history taking, clinical examination. Laboratory studies included complete blood count, liver and kidney function tests.

Lumbar puncture

CSF was collected from the subarachnoid space by a sterile spinal needle (*25* or *27* G) between the fourth and the fifth lumbar vertebrae. The CSF was collected in 3 sterile, screw-capped tubes. Two ml of CSF was collected in each tube. The first tube was labeled as No 1 (for direct Gram' stain, standard bacteriological

culture methods and antibiotics sensitivity testing). The second tube was labeled as No 2 for physical (color, aspect), chemical (glucose level, protein concentration and cytological examination). The third tube was used to test CSF with reagent strips (Sharma et al., 2021).

Blood culture and antibiotic sensitivity test

An aseptic technique was used to collect the blood from patients. The top of the culture bottle was wiped using an ethanol swab, and 10-12 ml of blood was taken (Giuliano et al., 2019).

Testing the CSF with rapid reagent strips

Combur-10 (Roche) reagent strip is a 10-patch test strip that is used to test urine for specific gravity, pH, leukocytes, nitrites, protein, glucose, ketones, urobilinogen, bilirubin, and blood. These test strips were used in this study to measure CSF protein, glucose and leukocytes. A separate CSF sample was used and according to manufacturer instructions, the reagent strip was dipped directly into the tube for approximately one second making sure that all test areas are moistened. When withdrawing the test strip, its edge was wiped against the rim of the vessel to remove excess fluid. After 60 s (60 - 120 s for the leukocyte test area) the color change was red against the standards provided (Moosa et al., 1995).

CSF analysis

The specimen number one was centrifuged at 2000-3000 rpm for 20 min. The supernatant aspirated with a sterile pipette, leaving approximately 0.5 ml of fluid in the specimen tube (supernatant can be reserved for biochemical studies), the sediment shaken to re-suspend.

Physical characteristics of CSF: Color and aspect.

Chemical analysis of CSF: Glucose: negative < 25, 1+ 25 - 75, 2+ 76 - 200, 3+ 201 - 650 and 4+ > 650 mg/dL, Protein: negative < 15, 1+ (15 - 65, 2+ 66 - 300, and 3+ >300 mg/dL and Lactate.

Cytological examination: The presence of leukocytes was graded: negative < 10, 1+ 10 - 50, 2+ 51 - 290, and 3+ > 291 cells/µl (Hrishi and Sethuraman, 2019).

Microbiological examination: Direct smears stained by Gram were done according to microbiological standards. Microscopical examination of Gram-stained smears was performed. Ziehl Neelsen (ZN) staining was done when indicated clinically and according to microbiological standards (Gray, 1992).

Culture of CSF: Inoculation was done on blood, chocolate and Mac-Conkey's agar plates and incubated aerobically and anaerobically in presence of 5-10% CO_2 at 37°C for 72 h. Identification of bacterial colony by colony morphology and biochemical reactions e.g: Coagulase test, Catalase test, Optochin test and bile solubility test, Indole production test and Oxidase test. Analytical Profile Index (API) 20 strep (BioMerieux, Germany) and (API) 20E (Bio-Merieux, France): an identification system using standardized miniaturized biochemical tests, used for biotyping and delineation of different species (Leazer et al., 2017).

Antibiotics sensitivity testing: Disk diffusion Kirby-Bauer method was used to determine the susceptibility of the recovered clinical isolates to antimicrobial agents. The diameters of inhibition zones were measured in mm using a ruler and classified as either susceptible, intermediate, or resistant to the agents that were tested (Assegu et al., 2020).

Brain MRI

MRI may be performed before the lumbar puncture in patients with neurological deficit, seizure, Glasgow score < 11.

According to the results of the above studies and discharge diagnosis, patients were categorized into three groups

Meningitis patients, 240 patients: presented with fever, meningeal symptoms and altered mental status, and with an abnormal number of white blood cells in CSF.

Encephalitis patients 30 patients: presented with acute onset of fever and a change in mental status and/or new onset of seizures, and a clear CSF, leukocyte count < 80/mm^3, all were lymphocytes or without CSF pleocytosis with no identification of bacteria by CSF culture or gram stain, and after exclusion of other causes of decreased mentation.

Meningism patients are 80 patients: presented with signs of meningeal irritation but CSF examination was normal and the subsequent investigations and evolution of the disease revealed the true diagnosis (Dian et al., 2020).

Confirmed meningitis patients were classified into two groups

Group I (bacterial meningitis): 110 patients with a positive Gram stain and/or CSF culture or positive blood culture with concurrent meningitis; or detection in the CSF of >100 white blood cells per ml.

Group II (aseptic meningitis): This group included 130 patients with CSF pleocytosis (≤100 WBCs), negative Gram stain; and the CSF and blood cultures were negative for bacterial meningitis.

Inpatient follow-up and outcome assessment

Patients were evaluated daily for symptom improvement or occurrence of new symptoms. Vital signs were assessed every four hours for the first 48 h and based on the need thereafter. Daily follow-up with a neurosign chart that included the following variables: Glasgow coma scale (GCS), seizures, headache, and nuchal rigidity was done during the inpatient treatment.

Patients were also assessed at discharge for gross neurologic deficits (visual problems, hearing deficits, and body weakness) and mini-mental state examination. Detailed analysis of epidemiologic characteristics, clinical data and laboratory findings, the causative organisms, prognosis of all the patients with BM were studied.

Statistical analysis

Data was collected and tabulated. IBM's SPSS statistics (Statistical Package for the Social Sciences) for windows, version 25, 2017 was used for statistical analysis of the collected data. Shapiro-Wilk test was used to check the normality of the data distribution. All tests were conducted with 95% confidence interval. P (probability) value < 0.05 was considered statistically significant. Charts were generated using SPSS' chart builder and Microsoft Excel for windows 2019.

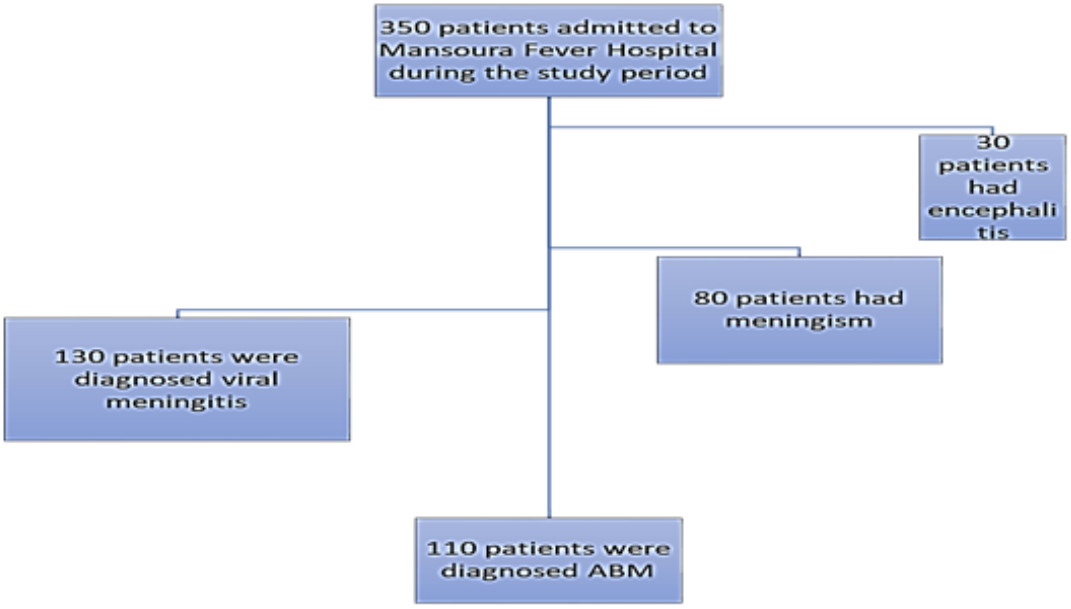

Figure 1. Flow chart of all studied cases (*350* patients).Classified into groups according to the causative pathogens and clinical presentation of acute meningitis.

Quantitative variables were expressed as mean and standard deviation, median, inter-quartile range, minimum and maximum values as appropriate while categorical variables were expressed as frequency and percentage. Cohen's kappa (κ) was run to measure reliability and agreement between the studied diagnostic methods and BM diagnosis.

RESULTS

This study included 110 patients with confirmed diagnosis bacterial meningitis (BM). BM occurred in males more than females. It affected patients from rural much more commonly than patients from urban communities (Figure 1).

The clinical presentation of BM was characterized mainly by fever (90.9%) and headache (88%). Signs of meningeal irritation that is, neck rigidity (90.9%), Kernig sign (72.7), and Brudziniski sign (69%) are significantly higher in patients with meningitis. Once neck rigidity, headache, or both are present with fever meningitis is highly suspected.

Antecedent illnesses (that is, diseases diagnosed at the time or shortly before the diagnosis of meningitis) were found in 74.5% of patients with BM. Pneumonia was the most common predisposing condition. A large percentage of meningitis patients, 33.6% reported a positive history of antibiotic intake in the few days before admission to the hospital**.**

The study shows that 79.1% of BM patients had a CSF leukocyte count in the range of > 100 up to 1,000 cell/mm^3 and 80% of BM patients had CSF neutrophil percentage > 50%. 85.5% of BM patients had elevated CSF protein (> 80mg/dl). While 81.8% of BM patients had decreased CSF glucose < 50 mg/dl. It was found that 90% of BM patients had elevated CSF lactate> 26 mg/dl. Out of 110 CSF samples, 95 cases, 86.4% of pathogens were detected by direct Gram-stained smear. Gram positive bacteria were detected in 55/110, 50% of cases. Gram negative bacteria were detected in 40/110 (36.4%) of cases. Gram positive *cocci* was the commonest 25/110 microorganism isolated in the present study. *S. pneumonia* was the most common isolated pathogen accounting for 24% of BM (Table 1).

Table 2 shows rapid reagent strip and CSF examination showed a positive correlation with laboratory test results that include, laboratory cell, protein and sugar values in cases of BM. This study shows a sufficient degree of agreement between CSF and blood culture results. Out of 110 patient 73 (66.4%) had blood culture growth of the same organism as the CSF culture. Furthermore, 23(20.9%) of patients who had positive CSF cultures and negative blood cultures.

BM carried a high mortality rate (22.7%). These include: One infant 6 m, one female patient 40 years old, one girl 16 years old, and all other cases were above 55 years old. Complications were found in 21.8% of BM patients who developed permanent sequelae such as hearing deficits, paralysis, cognitive slowness and memory trouble. Predictors of poor outcome of BM are CSF/serum glucose ratio >0.6, CSF protein >80 mg/dl and Tonsillitis (Figure 2 and Tables 3 and 4).

Table 1. CSF markers that could indicate BM.

CSF markers		Frequency	Percentage	Sensitivity (%)
CSF WBCs	**100- 1000**	87	79.1	100
	>1000	23	20.9	
Neutrophil	<50	20	18.2	81.8
	>50	90	81.8	
CSF glucose	<50 mg/dl	90	81.8	81.8
	>50 mg/dl	20	18.2	
CSF/serum glucose ratio	<0.6	97	88.2	88.2
	>0.6	13	11.8	
CSF protein	<80 mg/dl	16	14.5	85.5
	>80 mg/dl	94	85.5	
CSF lactate	>26 mg/dl	99	90.0	90
	<26 mg/dl	11	10	

Table 2. Frequency of pathogens causing BM detected by CSF direct stained film.

Direct stained film	Frequency	Percentage	Sensitivity (%)
Gram positive cocci	25	22.7	86.4
Gram positive diplococci	20	18.2	
Gram positive bacilli	5	4.5	
Gram positive chain	5	4.5	
Gram negative diplococci	17	15.5	
Gram negative coccobacilli	13	11.8	
Gram negative bacilli	10	9.1	
No detected strains	15	13.6	

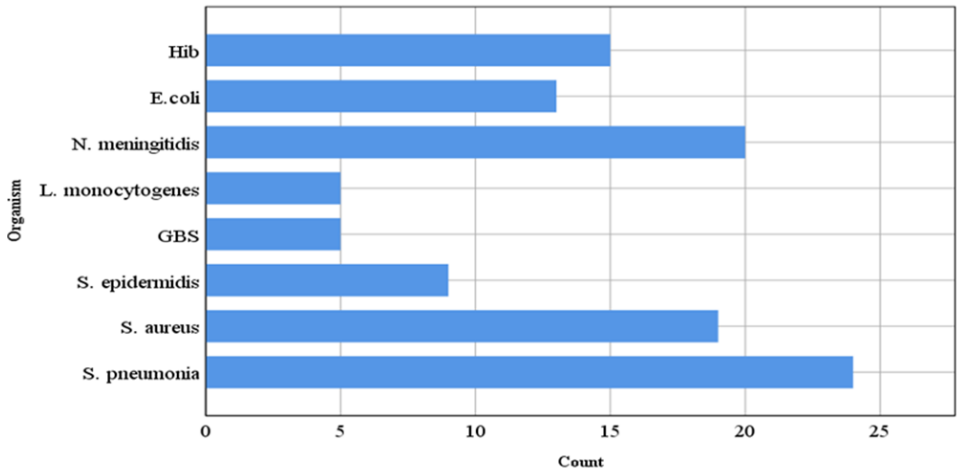

Figure 2. The frequency of bacteria causing BM by cultures.

Table 3. Correlation between reagent strip and laboratory cell, protein and sugar values in cases of ABM.

Variable	Number of correlated values	Number of non-correlated values	Sensitivity (%)	Kappa	p
Cell value	95	15	86.4	0.94	< 0.001
Protein value	90	20	81.2	0.819	< 0.001
Glucose value	86	24	78.2	0.819	< 0.001

Table 4. Predictors of poor prognosis in the studied cases of ABM.

Variable	R2	Exp(B)	Wald	P
CSF/serum glucose ratio >0.6	0.067	0.193	4.269	0.039
CSF protein >80 mg/dl	0.084	0.216	6.200	0.013
Tonsillitis	0.082	10.00	4.480	0.034
P is significant when < 0.05.				

DISCUSSION

In this study, 350 patients admitted to Mansoura Fever, Egypt with clinical features suggestive of meningitis. They underwent lumbar puncture, BM was diagnosed in 110 patients (31.43%), viral meningitis in 130 (37.14%), meningism in 80 (22.86%) cases and encephalitis in 30 (8.57%). The ratio of bacterial to aseptic meningitis cases vary between different studies. Abdelkader et al. (2017), found that the majority of meningitis cases over the two years period were possibly viral meningitis (42.86%) and bacterial meningitis cases with positive culture growth were (5.08%). However, in the study conducted by Amarilyo et al. (2011), bacterial meningitis was diagnosed in 10.3% of meningitis patients and 89.7% were found aseptic. This difference in the percentage of bacterial versus aseptic meningitis can be attributed to differences in the place and time of studies done. In developed countries, bacterial meningitis became less common in relation to viral meningitis especially after the implementation of anti-capsular vaccines.

In our study males were affected (61.8%) more commonly than females (38%). Our results are in agreement with the study of Abdkader et al. (2017), males were 44 (64.7%), compared to 24 who were females (35.3%). Our data showed that BM occurred more in rural than urban areas. This is in agreement with the study done by Afify et al. (*2007*) who demonstrated that 74.5% of BM patients had antecedent illnesses. Pneumonia was the most common predisposing condition in (33.6%) of patients. This is in agreement with the study of Brouwer et al. (2012), who showed that ear, sinus, or lung infections precede pneumococcal meningitis in 40% of patients.

Clinical features give clue to the early diagnosis of BM by a physician. In the current study the classic clinical features of bacterial meningitis include fever (90.9%), headache (88%), stiff neck (90.9%). Similar rates were reported by several investigators (Afifi et al., 2007; Arda et al., 2008). Kernig and Brudziniski signs were present in this study among (*72*.7 and 69% for each sign respectively. Another study on adults reported sensitivity values for Kernig's sign (*36%*) and Brudziniski's sign, 39% (Elmore et al., 1996). Furthermore, in a considerable number of meningitis patients, the above clinical features were absent (Heydarian et al., 2014; Dyckhoff-Shen et al., 2021). Prognostic factors that were associated with poor outcomes include old age≥ *65* years, neurological complications and late administration of antibiotics for patients with bacterial meningitis. From the above discussion it became evident that none of the symptoms and signs could accurately discriminate between patients with meningitis from those without it. Patients with suspected meningitis on clinical grounds should be referred for lumbar puncture and CSF examination as gold standard diagnostic tests (Brouwer and van de Beek, 2015).

In this study, 110 patients who showed clinical features of meningitis, 20.9% of these patients had a CSF leukocyte count >1,000 cells/mm^3, and, 79% had a leukocyte count > 100 - 1,000 /mm^3 with 100% sensitivity. Also, this study demonstrated a neutrophil dominance of CSF leukocyte count > 50% in 81.8% of bacterial meningitis patients with sensitivity of 81.8%. These results were documented in other studies (Martinot et al., 2018). This large number of patients with CSF leukocyte count less than 1,000/mm^3 79% may be a reflection of the high rates of antibiotic use before hospital admission that reached 60% resulting in partially treated meningitis.

This study showed that 81.8% of patients with BM had decreased CSF glucose concentration (glucose concentration < 50 mg/dl). Also, there was a significant increase of CSF protein in bacterial meningitis patients. Most studies reported the same findings (Martinot et al.,

2018). The CSF-blood barrier disruption causes a high CSF protein level in patient with meningitis (Julián-Jiménez and Morales-Casado, 2019).

Studies in adults have indicated that adding CSF lactate to routine CSF examination is better for the diagnosis of BM in a very short time (Alfred et al., 2021). The mechanism of increased lactate concentration in the CSF of patients with BM meningitis is not clear, but it may be due to increased anaerobic glycolysis of brain tissue due to a decrease in cerebral blood flow and oxygen uptake (Shamliyan, 2018; Xiao et al., 2016). In the present study, 99 (90%) patients with BM had elevated CSF lactate level, and 11(10%) had normal lactate with sensitivity of 90%. Many other studies showed the same result (Nazir et al., 2018; Shamliyan, 2018; Viallon et al., 2011). A meta-analytic study that included 404 neurosurgical patients revealed that SCF lactate levels were highly sensitive and specific for diagnosing bacterial meningitis (Houri et al., 2017).

In the current study, out of 110 specimens; using Gram stain 55 (50%) were found to be Gram positive and 40 (36%) were found to be Gram negative. From 55 Gram positive isolate, 24 were *S. pneumococca.* 19 *Staphylococcus aureus,* 9 *S. epidermidis,* 5 *GBS,* and *5 L. monocytogenes*. Different isolates were found, of 40 Gram negative isolates, 20 were *N. meningitides*., *15 Hib*, 13 *E. coli*. Comparing our results with Abdelkader et al. (2017), 48 isolates (67.6%) were found to be Gram-positive and 23 isolates (32.4%) were Gram-negative. Among 48 Gram positive isolates the majority were *S. pneumonia*.

Another study by Afifi et al. (2007) that was also conducted in Egypt found that *S*. *pneumoniae* was the leading cause of bacterial meningitis. This reflects a change in disease epidemiology since *N. meningitides* was for a long time the most common pathogen causing bacterial meningitis. Other several studies have reported high prevalence of *S. pneumonia* around the world (Jones et al., 2004; Owusu et al., 2012; Nasiri et al., 2019). *Meningococcus* was considered as the second common cause of BM in this study. This is in agreement with study of Kerstin et al. (2018) and WHO report (2017).

Urine reagent strips that measure glucose, protein, leukocytes and other urinary biomarkers have been used to evaluate CSF in several previous studies. In this study, the number of regent strips results coincide with the laboratory findings in *95* out of *110* patients (with sensitivity *86.4*%) for leukocytes, *90* out *of 110* (with sensitivity 81.2%) for protein*, 86* out of *110* (with sensitivity *78.2*%) for glucose. A higher rate of agreement between regent strips and laboratory results was also reported by Mazumder et al. (2018), Kumar et al. (2015) and Phillips et al. (2019). Another study found that increased CSF protein was increased in study group as compared to control group while cell count was significantly insensitive but its specificity was 63.79%. Sa˘glam et al. (2013).

Since bacterial meningitis can occur secondary to underlying bacteremia from other sources, so routine ordering of blood cultures in suspected casesis crucial for accurate diagnosis (McGill et al., 2016). In this study, blood cultures were done in all cases (*110),* it was reported that 73 (66%) were positive by the same bacteria present in CSF culture while 14 (12.7%) were positive by different isolates. However, 23 (20.9%) of blood cultures were negative. This is in agreement with results of Troendle (2019), demonstrated that blood cultures detect causative organism in 71% of cases. Blood cultures helped to identify the causative organism in 50-80% of pediatric and adult cases. The yield of blood cultures decreases by 20% if the patient has been pretreated by ntibiotics (Troendle and Pettigrew, 2019).

Bacterial meningitis is still a life-threatening disease that is associated with significant mortality and morbidity. In the current study the reported mortality rate of BM was 22.7% and about 21.8 % of BM patients developed permanent sequelae such as hearing deficit, paralysis, cognitive slowness in the form of delayed processing speed and memory troubles (Liu et al., 2012, Olbrich et al., 2018). This is in agreement with results of Troendle et al. (2019). The mortality rate was 31%. Without any treatment, the case-fatality rate can reach 70%, and one in five survivors of bacterial meningitis may be left with permanent disability including hearing loss, neurologic disability, and cognitive dysfunction (Gudina et al., 2018; Tsai et al., 2019).

Conclusions

The clinical symptoms and signs were inadequate to make a definite diagnosis of bacterial meningitis. Lumbar puncture and CSF examination is the gold standard method for diagnosing bacterial meningitis and is necessary to be done as soon as possible.

Reagent strip can be used reliably in CSF examination where laboratory facilities are limited or even routinely done in all hospitals for early diagnosis of bacterial meningitis.

The frequencies of the most common infectious agents causing BM in this study are *S. pneumonia, N. meningitidis* based on bacterial culture. Predictors of poor outcome of BM are CSF/serum glucose ratio >0.6, CSF protein >80 mg/dl and Tonsillitis respectively.

The use of traditional methods for diagnosing bacterial meningitis is time-consuming and has low sensitivity. So, the search for novel accurate and rapid molecular methods is necessary. The combination of both metagenomic Next Generation Sequencing mNGS and Whole Exome Sequencing WES may help to increase precision of diagnosis of bacterial meningitis particularly in neonates for guiding rapid effective therapeutic interventions. Progress in the field of predictive and

personalized medicine is also promising for improving the predictive power and accuracy for personalized antiobiotics treatment of BM.

Study strength and limitations:

Several studies were carried out internationally on the etiological and prognostic factors of bacterial meningitis yet, there are few studies conducted in Egypt. Up to the best of our knowledge, this study is one of few important studies that is carried out on the occurrence of bacterial meningitis in our hospitalized patients. Therefore, the strength of the current research is the study of the potential etiological and prognostic factors in those patients. Our study has some limitations that need to be acknowledged. The sample of patients was only conducted from a single center (Mansoura fever hospital) at a single time point. A small study sample reduces the power of event-free survival analysis, and the results obtained thus cannot be generalized globally to all Egyptian patients. These data can only be used to generate hypotheses that can be used in the future for a confirmatory study, that is, a mega randomized clinical trial. Since this is an observational study, many different techniques that can be applied to prevent or control for confounding could not be used. Moreover, the study did not consider the socioeconomic variables that significantly influence bacterial meningitis.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Fungal contamination of dental appliances: A cross-sectional study

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Orthodontic treatment makes necessary to use a longtime fixed-band appliance, it offers suitable conditions for fungal growth, dentures also allow fungal contamination on their surfaces. The aim of our study is the identification of the fungal species colonizing surfaces of dental appliances, the cross-sectional study was carried out on 60 patients wearing dental appliances, during 5 months, and sterile swabs were used and inoculated into Sabouraud's dextrose agar tubes. Yeast identification has been based on germ tube test, chlamydoconidia production and biochimical tests (Auxacolor, Api 20 C). The overall prevalence of fungal contamination of dental orthodontic appliance was 35%. The prevalence rate was 40% among patients who had dental appliances for 1 to 2 months, and 45,5% fort patients brushing their teeth at least twice daily, but no statistical relation with mycosis was found. Pain, burning and discomfort were correlated to fungal carriage p=0,038; OR=4,867 IC 95% (1.09-21.74). The fungal species most recovered were *Candida parapsilosis* 21.47%, *Candida Zeylanoides* 21.47%, *Candida albicans* 17.38%, and *Cryptococcus terreus* 13.04%. Mycological monitoring of dental prosthesis is crucial to prevent possible fungal's adverse health effects.

Key words: Orthodontic appliances, dentures, fungal contamination, *Candida* sp, *Cryptococcus terreus*.

INTRODUCTION

In recent years dental infections and biosecurity attracted greater interest of health professionals due to the spread of infectious diseases such as AIDS and Hepatitis B (Moreira et al., 2016). Oral appliances are divided in prosthodontics and othodontics, ranging from braces and retainers to dental crowns, fillings and dentures.

Denture stomatitis, is a common inflammatory lesion, the erythema is localized to the fitting surface of the

denture bearing areas of maxillary removable dental prosthesis (Neville et al., 2009). At one time oral fungal infections were a relatively uncommon event, but with advances in health care and an increasingly aging population, oral fungal infections are becoming more common. Stomatitis appears to be multiparametric; old age and concomitant decline of the immune defences, systemic diseases, smoking, ill-fitting denture and poor oral hygiene resulting in the accumulation of plaque on the dentures have all been proposed as predisposing factors (Barbeau et al., 2003; Grimound et al., 2005; Figueiral et al., 2007). The use of oral appliances is usually asymptomatic; however, it may cause mild soreness or burning sensation.

Fixed orthodontic treatment can lead to alteration in the oral environnement. A study revealed that orthodontic treatment is correlated to an increase in the count of microorganisms, an increased accumulation of plaque, and a decrease in the level of pH, which leads to a major risk of caries and periodontal diseases (Gujar et al., 2020). Orthodontic treatment leads to significant changes in both supragingival and subgingival microbial flora showed that there was an increase in the level of subgingival pathogens after the placement of orthodontic appliances (Gujar et al., 2020).

Numerous fungi are potentially pathogenic microorganisms, *Candida albicans* is the principal species associated with human infections ranging from the more common oral thrush to fatal systemic superinfections in patients who are afflicted with other diseases (Krishnan, 2012). Besides *Candida* sp, other fungi can cause oral diseases in humans: *Aspergillus, Cryptococcus, Histoplasma capsulatum, Blastomyces dermatitidis, Zygomycetes class, Coccidioides immitis, Paracoccidioides brasiliensis, Penicillium marneffei, Sporothrix schenckii,* and *Geotrichum candidum* (Neville et al., 2009; Samaranayake and MacFarlane, 1990). This study aimed identifying species's colonizing dental appliances surfaces.

MATERIALS AND METHODS

This cross-sectional study was conducted among patients wearing dental appliances, 60 patients were submitted to a detailed clinical interview and oral clinical examination, and were instructed to keep their dentures. After obtaining informed consent and before collecting samples, a questionnaire was performed among dental wearers. From literature various factors may contribute to increase or decrease in fungal growth these may include oral hygiene of patient, immune status of patient and the medication patient is taking. So, all these factors were considered in this study. Sixty patients wearing dental appliances were selected according to following criteria: Patient included were clinically healthy; they had good oral hygiene and clinically normal oral mucosa, and had no any kind of medication, the study received approval from the ethical committee.

After obtaining informed consent and before collecting samples, a questionnaire was performed among oral appliances wearers; it included (presence of carries, pain, or odour, teeth brushing per day, toothbrushing duration, prior treatment, type of appliance). Swabs were taken according to a standard protocol. Samples were taken of each appliancee surface by means of sterile swabs (that is, each was left in place for 30 s), samples were obtained by swabbing a region (1 × 5 cm line) of the anterior flange of the appliance. Orthodontic appliances were assessed using sterile swabs on the metallic braces (Figure 1).

All samples were inoculated on Sabouraud dextrose agar (SDA) medium and incubated at 30°C for 7 to 14 days, to allow filamentous fungi and yeasts to grow. Identification of species was performed by phenotypical tests (germ tube formation, chlamidoconidia production), and with the aid of commercially available systems like biochemical carbohydrate fermentation and assimilation using Api 20c and Auxacolor. Data were managed and analyzed using statistical software 17.0 (SPSS, Inc., Chicago, IL).

RESULTS

The 60 patients using dental appliance enrolled in this study had a mean age of 48 ± 2.62 years, the age range of the participants was between 10 and 79. The 60 dental appliances were composed of 34 prosthodontics (dentures) and 26 orthodontics appliances (braces). The overall prevalence of fungal contamination of dental appliances was 35% (21/60). The specific prevalence of fungal contamination of denture was 38% (13/34), and the fungal contamination of braces was 31% (8/26). Dentures were the most contaminated appliances. Pain, burning and discomfort were correlated to fungal carriage p=0.038; OR=4.867 IC 95% (1.09-21.74) (Table 1).

Yeasts of Candida genus were present in 20 (86.96%) of positives appliances. *Candida parapsilosis* and *Candida zeylanoides* were the species most recovered in this study (21.74% respectively). The various species are outlined in Table 2. Two samples were polymicrobials with the concomitant presence of *C. parapsilosis* and *C. zeylonoides.*

DISCUSSION

Denture-induced stomatitis is an inflammatory reaction of the denture-bearing mucosa that affects approximately 65% of complete upper denture wearers (Webb et al., 1998), in our study fungal contamination was more important among dentures.

Fungi and protozoa may find favorable conditions to thrive in dental units. Literature papers have reported

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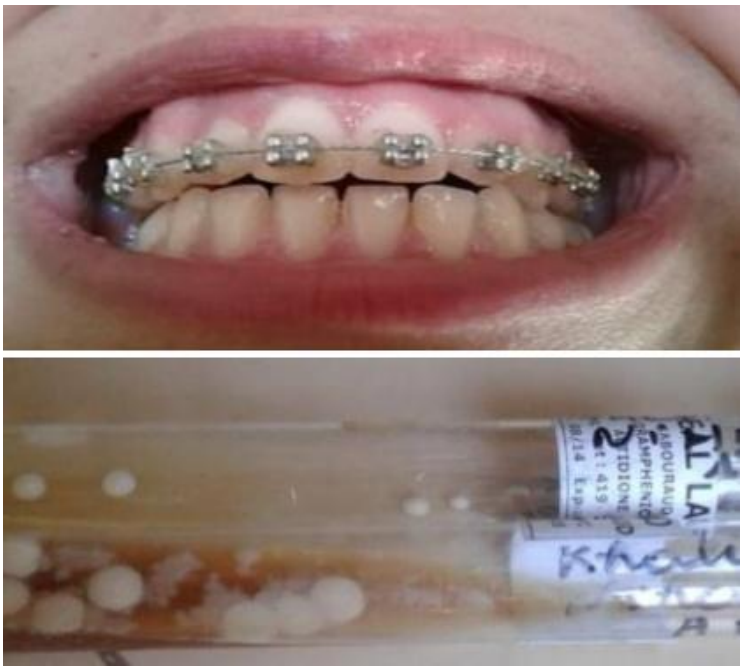

Figure 1. Orthodontic appliance infected by *Candida albicans* strain.

Table 1. Individual risk factors and their correlation with appliances fungal carriage.

Variable	Fungal +	Fungal -	OR	IC 95%	P
Age	-	-	-	-	NS
Sex M/F	8/13	15/24	1 .161	0 .341-3 .026	NS
Nbr brushing per day 1-2/more than 2 times	-	-	1 .563	0 .497-4 .913	Ns
Caries +/-	16/5	31/8	0 .826	0 .232-2 .940	NS
Pain +/-	18/3	25/14	4 .867	1 .09 -21 .74	0 .038
Odour+/-	-	-	0 .747	0 .253-2 .207	NS
Residence urban/rural	13/8	17/22	0 .464	0 .143-1 .502	NS
Toothbrushing duration Less than 1 min/more than 1 min	-	-	0 .800	0 .251-2 .545	NS
Prior treatment +/-	13/8	23/16	1 .131	0 .381-3 .354	NS
Type of appliance	-	-	1 .525	0 .773-3 .009	NS

Table 2. Fungal species recovered from dental appliances surfaces.

Species	N	Percentage
Candida parapsilosis	5 (3 dentures, 2 braces)	21.74
Candida zeylanoides	5 (2 dentures, 3 braces)	21.74
Candida albicans	4 (1 denture, 3 braces)	17.38
Cryptococcus terreus	3 (2 dentures, 1 braces)	13.04
Candida kefyr	1 (1 denture)	4.35
Candida pelliculosa	1 (1 denture)	4.35
Candida rugosa	1 (1 braces)	4.35
Candida famata	1 (1 denture)	4.35
Candida boidini	1 (1 denture)	4.35
Candida glabrata	1 (1 denture)	4.35
Total	23	100

microorganism counts ranging from 100 to 400.000 CFU/ml in dental units (Kim et al., 2000; Ozcan et al., 2003). It is usually asymptomatic; however, may cause mild soreness or burning sensation in some (Krishnan, 2012), we found a strong correlation between fungal presence and soreness or burning sensation (p=0.038); OR=4.867 IC 95% (1.09-21.74). Poor hygiene of dentures is also associated with fungal stomatitis (Grimound et al., 2005; Budtz-Jörgensen, 1978). Disinfection of dentures and dental appliances plays a key role in eradicating this form of candida infections (Muzyka, 2005), but number of brushing per day and duration of brushing teethes was not linked to presence of fungi in our study.

In a previous study, the duration of denture use was ranging from 3 weeks to more than 40 years, was generally unrelated to degree of contamination (Glass et al., 2001), in concordance with our results. On the other hand, Gujar et al. (2020) found that the microbial contamination in metallic brackets was higher than that of aligners, when used for a month. The porosity of the acrylic allows fungal and bacterial contamination throughout the entire denture (Glass et al., 2001). Moreover, the relatively acidic and anaerobic microclimate underneath the denture provides an ideal environment for yeast growth, and the denture shields the mucosa from the saliva and its local immunity-enhancing properties. Thus, the denture or any acrylic-containing dental appliance can serve as a source of inoculation for bacteria and fungi (Muzyka, 2005).

Candida species may be recovered from up to one-third of the mouths of normal individuals and are considered inhabitants of the normal flora of oral and gastrointestinal tract (Krishnan, 2012). However, the involvement of Candida as the main causative agent in denture-induced stomatitis (Pinto et al., 2008) was first described by Cahn in 1936, *Candida albicans* remains the most frequently isolated fungal in the oral cavity but other species have also been isolated and involved in disease (Garg et al., 2012), *C. albicans* occurred in the third place. The most commonly occurring dental fungal infection is caused by Candida species with *C. albicans* most often encountered (Muzyka, 2005), this is in accordance with our results.

Filamentous fungi can also be considered as biofilm-forming organisms because they are well adapted to grow on both biotic and abiotic surfaces. The formation of biofilms (Damasceno et al., 2017), but we did not recorded any filamentous fungi in this study.

In vitro adherence studies illustrate that *C. albicans* attaches readily to various resins, glass, and metal surfaces. The ability of *C. albicans* to adhere to polymeric surfaces has been correlated with attractive hydrophobic and repulsive electrostatic forces (Garg et al., 2012).

In denture wearers, Candidiasis is aggravated by the adhesion of *C. albicans* to the tissue surface of the maxillary denture base, which serves as an effective reservoir of microorganisms (Garg et al., 2012).

Of the organisms recovered in this study, *C. parapsilosis, C. famata*, *C. rugosa* and *C. glabrata* are most frequently involved in human infections. Species that were once considered to be saprophytic are becoming more commonly associated as opportunists causing human disease.

In another study, Candida species isolated from complete upper denture wearers were *C. albicans, C. glabrata, C. parapsilosis, C. guillermondii, C. krusei, C. lusitaniae, C. tropicalis* (Pinto et al., 2008), *Candida kefyr* and Cryptococcus were involved in oral fungal infections (Krishnan, 2012).

Basically, the present analysis has pointed to a high prevalence of fungal organisms in oral appliances, in another study samples obtained at both peri-implantitis (31.6%) and healthy (40%) implant sites were dominated by *Candida* sp (Schwarz et al., 2015), which is in accordance with our results.

C. albicans, C. boidinii, and *Rhodotorula* sp have already been identify by Polymerase chain reaction (PCR) in oral peri-implantis sites (Schwarz et al., 2015), *C. glabrata* was also found in denture appliance (Glass et al., 2001).

C. boidinii is widespread and it has been isolated from diverse substrate related to human activity (wine fermentation, olive manufacturing, tepache), and natural environments (soil, seawater, sap fluxes of many sugar rich tree species (Camiolo et al., 2017).

Cryptococcosis also infects the central nervous system, skin and the oral mucous membrane (Glick et al., 1987), it can produce a variety of cutaneous and oral mucosal manifestations such as superficial ulcers, nodules, granulomas or carcinoma-like lesions (Myrvik and Weiser, 1988). Intraoral sites commonly affected are gingiva, palate and tooth socket after extraction. Violaceous nodules of granulation tissue, swellings and ulcers are the various forms of oral lesions reported (Samaranayake and MacFarlane, 1990). The numerous opportunistic and pathogenic microorganisms found in this study were unexpected and are known to produce not only substantial oral infections, but also systemic diseases as mentioned in previous studies (Pinto et al., 2008). Furthermore, the present analysis failed to identify any significant correlation of either fungal organisms or disease severity with risk factors, such as caries and teeth brushing.

Conclusion

Candida species are assumed to cause disease by direct tissue invasion, either by inducing a hypersensitive state or by producing potent Candida toxins. The use of oral appliances is known to increase the carriage of Candida in healthy patients, and the proliferation of *Candida* sp strains can be associated with denture-induced

stomatitis. Being aware of these fungal risks, disinfection of dental appliances must be reinforced, and it is desirable to institute procedures to minimize the number of potentially fungal pathogenic agents.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Diagnostic value of two HIV rapid diagnostic tests 16 years after their adoption in national strategies in the Central African Republic (CAR)

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The broad genetic diversity of HIV requires regular reassessment of adopted HIV diagnostic tests. The objective of this study was to reassess the performances of Determine® HIV 1/2 and Uni-gold® HIV1/2, 2 HIV rapid diagnostic tests of the national algorithm, adopted since 2005 in Central African Republic. A reference prospective panel of 150 plasma samples were tested in duplicate with Determine® HIV 1/2 and Uni-gold® HIV, according to reference serological immune-enzymatic method. Analytical and virological characteristics were calculated. Sensitivity, specificity, positive and negative predictive values (PPV, NPV) of Determine® HIV 1/2 were 100%, as well as the specificity and NPV of Uni-gold® HIV 1/2. Uni-gold® HIV 1/2 sensitivity and PPV were 96 and 92%, respectively. The Cohen k coefficient was close to 1 for the 2 tests, 1 for Determine® HIV 1/2 and 0.97 for Uni-gold® HIV 1/2. Except for the sensitivity of Uni-gold® HIV 1/2, the performances of 2 tests were acceptable and in perfect agreement with the reference tests. These results require a revision of the algorithm. In addition, an exploration in molecular biology is indicated to determine the subtypes of the HIV strains of the 4 samples that tested false negative with Uni-gold® HIV 1/2.

Key words: HIV, rapid diagnosis test, sensitivity, specificity, Africa, Central African Republic.

INTRODUCTION

According to Joint United Nations Program on HIV/AIDS (UNAIDS) 2019 report, 38 million people were living with HIV/AIDS (PLHIV) and 32.7 million people have died from HIV/AIDS since the start of the epidemic worldwide. Sub-Saharan African countries are heavily impacted by HIV/AIDS, with two-thirds of PLHIV and HIV/AIDS-related

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deaths of all cases worldwide (ONUSIDA, 2020). To eliminate HIV infection by 2030, the UNAIDS ‘‘95-95-95’’ goals recommend that first, 95% of the HIV-infected population should know their HIV status; second, 95% of those who know their status should receive antiretroviral therapy (ART); and finally, 95% of patients receiving ART should have a suppressed viral load (undetectable HIV RNA <50 copies/mL) after 2020 (UNAIDS, 2015). Thus, to control the HIV disease and achieve its elimination, HIV diagnosis appears to be the first step. In high HIV epidemic burden and limited resource settings, such as sub-Saharan African countries, reliable and accurate diagnostic tests of HIV is therefore crucial for assessing HIV status and introducing patients to a continuum of cares. Indeed, misdiagnosis leads to inappropriate decisions which often delay the initiation of ARV treatment during this period of the ‘‘test and treat’’ strategy. Although WHO periodically evaluates HIV rapid diagnostic tests (RDTs) to indicate which ones are prequalified for use in the developing countries, the World Health Organization (WHO) still recommends evaluating the performance of tests under local conditions of use on the field before adopting them in national strategies and their widespread use (CDC, WHO, APL, 2001; WHO, 2016).

This is, for example, the case of the Central African Republic, a country of approximately 5 million inhabitants with a national seroprevalence among people aged 15 to 45 years of 3.6%, that is, a total of 120,000 people living with HIV (spectrum). The Determine® HIV 1/2 (Alere, Japan) and Unigold® HIV 1/2 (Trinity Biotech, Ireland), 2 rapid tests, were selected in 2005 after a study in a sequential HIV screening algorithm that had a sensitivity and specificity of 100% and over 98%, respectively (Ménard et al., 2005). Many studies have subsequently shown that the CAR is a country where HIV strains of wide genetic diversity circulate, which is also a dynamic phenomenon with more than 70% of CRFs and the appearance of subtypes that were absent there (Gody et al., 2008; Charpentier et al., 2012; Mossoro et al., 2017). This genetic variability could be the source of under-detection of certain strains (Mossoro et al., 2016). It then becomes crucial to recheck the performance of these tests 16 years later to guarantee the algorithm's effectiveness in detecting all HIV strains. The objective of this study was to assess the performance of Determine® (Alere, Japan) and Unigold® (Trinity Biotech, Ireland), 16 years after their adoption in national strategies.

METHODS

This is a cross-sectional study that took place in *Laboratoire National de Biologie Clinique et de Santé Publique in Bangui,* the capital city of the CAR, which became the national reference laboratory (LNR) of HIV, with the implementation of the molecular biology unit in 2011. Among other tasks, it is responsible for evaluating diagnostic tests. A reference panel of 150 samples, including 100 positive and 50 negative plasmas were prospectively collected, which had been tested according to reference national serological algorithm for HIV testing, using in parallel Genscreen® ULTRA HIV Ag-Ab HIV-1/2 Version 2 (Bio-Rad, Marnes-la-Coquette, France) and Murex® HIV 1.2.0 Ag/Ab Combination (Diasorin, Saluggia, Italy), as the gold standard. All plasma were frozen at -80°C until processing. Genscreen® ULTRA HIV Ag-Ab HIV-1/2 Version 2 (Bio-Rad, Marnes-la-Coquette, France) and Murex® HIV 1.2.0 Ag/Ab Combination (Diasorin, Saluggia, Italy) are 4th generation ELISA tests which contain recombinant HIV-1 and 2 capsid and surface proteins. The reference panel was further tested with Determine® VIH 1/2 (Abbott, Japan) and Uni-gold® VIH 1/2 (Trinity Biotech, Ireland), following the instructions of the manufacturers, by two clinical microbiologists blinded regarding the sample groups. Indeterminate readings were further read by a third microbiologist. Determine® VIH 1/2 (Abbott, Japan) and Uni-gold® VIH 1/2 (Trinity Biotech, Ireland) are 2 rapid immunochromatographic diagnostic tests whose reagents contain like ELISA reference tests, recombinant surface capsid and surface proteins of HIV-1 and 2. This alternative algorithm uses Determine® VIH 1/2 (Abbott, Japan Ag) rapid immunochromatographic test as a screening test and Uni-gold® VIH 1/2 (Trinity Biotech, Ireland) as a confirmatory test. For statistical analysis, sensitivity (Se) was calculated as the number of real positives divided by the sum of real positives plus false negatives. Specificity (Sp) was calculated as the number of real negatives divided by the sum of real negatives plus false positives. HIV-1 seroprevalence of 3.5% in general adult population of the CAR in 2019 (aidsinfo@unaids.org) was used in calculating positive predictive value (PPV) and negative predictive value (NPV), according to Bayes’ formulae, as follows (Collectif BioBayes., 2015):

PPV = sensitivity × prevalence/[sensitivity × prevalence + (1-specificity) × (1-prevalence)]

NPV= specificity × prevalence/[(1-sensitivity) × prevalence + specificity × (1-prevalence)]

The confidence intervals for each variable was calculated at 95% (95%CI) using a normal distribution. The 95% CI of the estimated sensitivities, specificities, PPV, and NPV were calculated using the formula: $f \pm 1.96\ [f\ (1\text{-}f)\ /n]^{1/2}$, where f is the sensitivity, the specificity, PPV, or NPV and n is the number of specimens tested. The Cohen’s coefficient κ (Cohen, 1960) was interpreted according to the Landis and Koch scale (< 0 as indicating no agreement, 0-0.20 as slight, 0.21-0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1 as near perfect agreement) (Landis and Koch, 1977). The study was approved by the Ethical and Scientific Committee, Faculty of Health Sciences, University of Bangui constituting the Institutional Ethical Committee.

RESULTS

The results of the analytical performances of Determine® VIH 1/2 (Abbott, Japan) and Uni-gold® VIH 1/2 (Trinity Biotech, Ireland) are depicted in Table 1. Among the 100 HIV samples known to be HIV-positive with ELISA algorithm, all were positive with Determine® VIH 1/2 (Abbott, Japan), 96 were positive and 4 were negative with Uni-gold® VIH 1/2 (Trinity Biotech, Ireland). All the 50 HIV samples known to be HIV-negative with ELISA algorithm were also negative with both rapid tests. Taken together, sensitivity and specificity of the Determine® VIH 1/2 (Abbott, Japan) were 100.0%, as well as the specificity of Uni-gold® VIH 1/2 (Trinity Biotech, Ireland).

Table 1. Sensitivities, specificities, positive and negative predictive values, and agreements between the 2 rapid tests and ELISAs algorithm.

Variable	Determine® VIH ½ (Abbott, Japan) (95%CI)	Uni-gold® VIH 1/2 (Trinity Biotech, Ireland) (95%CI)
Number of samples	150	150
True positive	100	96
True negative	50	50
False positive	0	4
False negative	0	0
Sensitivity (%)	100.0 (99.0-100)	96.0 (94.0-98.0)
Specificity (%)	100.0 (99.0-100)	100.0 (99.0-100)
Predictive positive value (%)	100.0 (99.0-100)	92.0 (90.0-94.0)
Predictive negative value (%)	100.0 (95% CI: 99.0-100)	100.0 (95% CI: 99.0-100)
Cohen k Coefficient	1.0	0.97

But the sensitivity of Uni-gold® VIH 1/2 (Trinity Biotech, Ireland) was 96.0% (95%CI: 94.0-98.0). The reliability of Determine® VIH 1/2 (Abbott, Japan) and Uni-gold® VIH 1/2 (Trinity Biotech, Ireland) estimated by the Cohen's k coefficient was 1 and 0.97, respectively, measuring the concordance between each rapid test and reference serological algorithm, demonstrating almost a perfect agreement (0.81-1) according to Landis and Koch scale. At HIV-1 seroprevalence of 3.5% in general adult population of the CAR in 2019 (UNAIDS, 2019), the PPV and NPV were 100% (95% CI: 99.0-100) for Determine® VIH 1/2 (Abbott, Japan), 92.0% (95% CI: 90.0-94.0) and 100.0% (95% CI: 90.0-100) for Uni-gold® VIH 1/2 (Trinity Biotech, Ireland), respectively.

DISCUSSION

The analytical performances of the sequential alternative algorithm which associates Determine® VIH 1/2 (Abbott, Japon) and Uni-gold® VIH 1/2 (Trinity Biotech, Ireland) in the Central African Republic (CAR) were herein evaluated. The results of this assessment showed that both tests had the specificity, NPV of 100% such as sensitivity and positive predictive value of Determine. In contrast, Unigold's sensitivity and PPV were 96 and 92%, respectively. The virological performances of HIV rapid tests in the Central Africa Republic are yet poorly established. The evaluation of virological performances of Determine® VIH 1/2 (Abbott, Japon) and Uni-gold® VIH 1/2 (Trinity Biotech, Ireland) was done in 2005 (Menard et al., 2005). In addition, Central Africa is characterized by the broad genetic diversity of HIV-1 strains (Gody et al., 2008; Charpentier et al., 2012; Mossoro et al., 2017) which can be associated with false negativity of HIV immunochromatographic rapid diagnostic tests (Aghokeng et al., 2009), and by a variety of factors which can be associated with false positivity or unspecific reactivities, including disturbances affecting the B cell-driven immunity during infectious diseases, such as marked immunological stimulation, strong nonspecific polyclonal B-cell activation, hypergammaglobulinemia, and production of circulating immune complexes (Klarkowski et al., 2014, 2013; Mbopi-Keou et al., 2014).

HIV rapid tests are more and more developed and used, particularly in resource-limited settings and may be of variable quality (UNITAID and WHO, 2018). In the present study, the analytical performances of Determine® VIH 1/2 (Abbott, Japan) and Uni-gold® VIH 1/2 (Trinity Biotech, Ireland) in the CAR, were evaluated using a collection of 100 positive and 50 negative sera randomly selected through the CAR HIV seroprevalence surveillance survey. The results showed excellent analytical performances of Determine® VIH 1/2 (Abbott, Japan) and Uni-gold® VIH 1/2 (Trinity Biotech, Ireland), except for the sensitivity of Uni-gold® VIH 1/2 (Trinity Biotech, Ireland), despite the risk of false-positive results with frequent inconclusive sera in this area of Africa (Klarkowski et al., 2013; Mbopi-Keou et al., 2014). The sensitivity of Uni-gold® VIH 1/2 (Trinity Biotech, Ireland) was 96.0% due to 4 positive samples with the reference tests which were negative with Uni-gold® VIH 1/2 (Trinity Biotech, Ireland). Finally, the analytical performances of Determine® VIH 1/2 (Abbott, Japan) were within the limits required by the WHO for HIV rapid tests (that is, sensitivity ≥99.0% and specificity ≥98.0% (WHO, 2016, 2017), likely allowing it to detect all HIV-1 strains circulating in the CAR. The sensitivity of the Uni-Gold HIV test (Trinity Biotech, Dublin, Ireland), which is low (96.0%) unlike the 2005 assessment (> 98.0%) (Menard et al., 2005) needs improvement. Previous studies showed similar results. In Tanzania, Determine® VIH 1/2 (Abbott, Japan) had high similar virological performances, sensitivity and specificity were 100% but specificity decreased (96.8%) when using whole blood (Kroidl et al., 2012). Likely, the sensitivity of Uni-gold® VIH 1/2 (Trinity Biotech, Ireland) was 81.9% in Australia (Keen et al., 2017).

This study has some limitations. In particular, the number of blood samples is limited. Thus, the number of negative and positive specimens to be analyzed should be sufficiently high; a higher number of 200 in each case is recommended by the French accreditation committee (Comité Français d'accréditation, 2015). The determination of the subtypes of the 4 negative samples with Uni-gold® VIH 1/2 (Trinity Biotech, Ireland) is indicated to understand the reasons for this negativity.

Conclusion

Taken together, the alternative rapid tests algorithm which associates Determine® VIH 1/2 (Abbott, Japan) and Uni-gold® VIH 1/2 (Trinity Biotech, Ireland) in the CAR must be revised, 16 years after adoption. Thus, it may be suitable for routine use in the general population of the CAR. Further studies are necessary to understand the reasons for the negativity of Uni-gold® VIH 1/2 (Trinity Biotech, Ireland) in the 4 samples.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Survey on efficiency of inoculation methods of *Pseudomonas fluorescens* on growth and yield of *Thymus kotschyanus*

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Thyme is one of the most important medicinal plants in wild rangeland in Iran that has lots of benefits. *Pseudomonas flourescence* is one of the effective plant growth promoting bacteria (PGPR) as a bioinoculant for medicinal plants. This study aims to survey three inoculation techniques with PGPR on growth and oil content in *Thymus kotschyanus* for organic cultivation. For this research, an experiment was conducted in Randomized Complete Block Design at Research Institute Forest and Rangelands, Tehran, Iran, with four treatments and three replications. In the first method, thyme seed was treated in liquid bacterial suspension for 3 h, in the second method bacterial suspension injected around the root before cultivation in farm and in the third method both seed and root have been treated by the bacterial suspension. In all three methods of inoculation of *P. fluorescence*, an increase in growth and the amount of essential oil was determined. The highest amount of root volume (30 mL) compared to the control (15 mL) was significant at the level of P=0.05 and the highest amount of essential oil (1.74%) was obtained by using the third inoculation method. It could be concluded that the microbial inoculation method has a great influence on the yield of *T. kotschyanus* and bacterial inoculation of seeds and roots had a greater effect, rather than inoculated separately for organic cultivation.

Keywords: Inoculation method, biofertilizer, thymus, plant growth promoting.

INTRODUCTION

The genus *Thymus* belongs to the family Lamiaceae and 18 species of this genus have been identified in Iran. There are approximately 350 different species of thymus worldwide. *Thymus kotschyanus* is one of the most important medicinal plants found in wild rangelands in Iran. Secretory hairs are the site of the accumulation of thyme essential oil (Salehnia et al., 2021).

All thymus species are rich in volatile compounds and mainly contain thymol and carvacrol, which are potent disinfectants. The essential oil of this plant has a special place in world trade. The best way to prepare essential oil from thyme is to distill it with water, which produces the

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highest yield of essential oil. Thyme contains 0.8 to 2.6% of essential oil, most of which are phenols, monoterpene hydrocarbons, and alcohols. Thyme contains compounds such as flavonoids, saponins, and bitter substances (Hedden et al., 2002).

Thyme aerial parts contain essential oils, tannins, saponins, and herbal disinfectants. Thyme leaves are used in food products, as well as plant essential oils in beverage, pharmaceutical and cosmetic industries.

Application of microorganisms as biofertilizers for improve crops and production has been used and become a common practice in the last years. Plant growth-promoting rhizobacteria (PGPR) (Kloepper, 1993) has been known as biofertilizer because these microorganisms adapt and grow rapidly around plant rizosphers (Hernández-Montiel et al., 2017; Azimova et al., 2012). However, some reports indicate that rhizobacteria inhibit the maximum growth of some plants by producing hydrogen cyanide. Research has shown that PGPR can increase plant growth and mineral uptake. They facilitate plants growth even in stressful situations (Salehnia et al., 2020).

The use of PGPR reduces the need for chemical fertilizers and pesticides for medicinal and aromatic plant species (Elavarasi et al., 2020; Amalan et al., 2017).

There have been numerous reports of stimulant effects of these bacteria in the production of more valuable plant chemicals and medicinal metabolites (Strigul et al., 2006; Salehnia et al., 2020).

Pseudomonas fluorescens can cause increase in the plant's access to absorbable iron in the rhizosphere and subsequently play an important role in improving plant growth in terms of quantity and quality (Ghorbanpour et al., 2014). They are famous biofertilizers and also through various mechanisms such as stimulating the production of plant hormones such as auxin, cytokine and gibberellin and also preventing the production of ethylene, increasing the solubility of inorganic and organic phosphate, producing microbial siderophores to increase plant access to absorbable iron, nitrogen fixation in symbiotic or non-symbiotic relationship. Jaleel et al. (2007) indicated a significant increase on the amount of ajmalicin by the application of non-native *P. fluorescens* on Vinca seedlings (del Rosario et al., 2017).

The beneficial effects of PGPR and the mechanism of joint adaptation of plants exposed to water deficit stress (WDS) are always related to the interactions of plants and microorganisms, which have exceptional effects on morphological and anatomical traits of roots, such as root networks and their biomass (Shahin et al., 2010; Shrivastava et al., 2014).

Phosphate solubilizing bacteria expand plant growth by absorbing essential minerals and increasing the solubility of phosphorus in low-soluble mineral phosphates such as phosphate rock, and many of them also release phosphorus from organic compounds by producing phosphatase enzymes (Messele, 2012).

Utilizing biofertilizers under the organic agricultural system is an approach to harvest high-quality and safe products from medicinal plants (Dawa et al., 2014).

This study aimed to increase the quantity as well as the quality of *T. kotschaynus yield using P. fluorescens* without chemical fertilizers. Evaluation of three microbial inoculation methods was carried out as well. This study was conducted at the Alborz Research Complex, Research Institute of Forests and Rangelands, in Randomized Complete Block Design (RCBD) with four treatments and three replications, on *T. kotschyanus* inoculated with *P. fluorescens*.

MATERIALS AND METHODS

In this study, to investigate the efficiency of three inoculation methods of *P. fluorescens* on growth, characteristics and percentage of essential oil of *T. kotschyanus*, an experiment was conducted in Randomized Complete Block Design with four treatments and three replications at Alborz Research Complex, Research Institute of Forests and Rangelands.

Farm soil characteristics

Phosphorus (ppm), 51/9; Potassium (ppm), 788/475; Organic matter (percentage), 1/878; Texture, Lumi Sandy; Acidity, 8/1.

Bacterial inoculum origin

The standard bacterial strain of *P. fluorescens* (169) was obtained from the Soil and Water Research Institute, Tehran, Iran.

Bacterial inoculation methods in thymus

Method1

Thymus seeds were placed in a sterile plate after determining the germination potential. For each seed, 5 mL of bacterial liquid suspension of *P. fluorescence* standard strain (169) prepared by the Soil and Water Research Institute with a population of 10^8 cfu/mL was added. For better effectiveness, Arabic gum as a carrier was added for seed adhesive. Bacterial suspension was inoculated at 10^8 cfu/mL at room temperature (25°C) on a shaker at 120 rpm. Then, after 48 h of incubation, constant turbidity with absorption of 560 nm was read by spectrophotometry.

Method 2

After rooting and seedlings emergence, 50 mL of the bacterial suspension was added with 10^8 cfu/mL using sterile syringe, in the zone around the root, in fact in the rhizosphere area of the plants. The suspension was prepared and the turbidity was fixed as in the first method.

Method 3

Both site inoculation, bacterial suspension with a population of 10^8 cfu/mL grown in B-King liquid medium was added by half McFarland method in Erlenmeyer 100 mL with physiological serum in a ratio of 1 to 9 to reach a population of 10^7 cfu/mL, then as in the

Table 1. Results of analysis of variance of growth parameters of *Thymus kotschyanus* under three inoculation methods of *P. fluorescens* compared to control group.

Treatment	Dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root volume (mL)	Number of branches	Branch height (cm)
Control	20^{ns}	2.75	1.87^{ns}	15^{ns}	20^{ns}	4.20^{ns}
P. fluorescens SRI	24.75^{ns}	3.55^{*}	2.82^{*}	30*	25^{ns}	6.5^{*}
P. fluorescens SI	22.45^{ns}	3.15^{*}	2.15^{ns}	21^{ns}	22^{ns}	5.05^{ns}
P. fluorescens RI	23.04^{ns}	3.21^{ns}	2.3^{5ns}	27*	23^{ns}	5.21^{ns}

p-value <0.05 *, <0.10**, <0.001***, <0.0001****, ns: no significant. RI: Root inoculation, SI: Seed Inoculation, SRI: Seed & Root Inoculation.

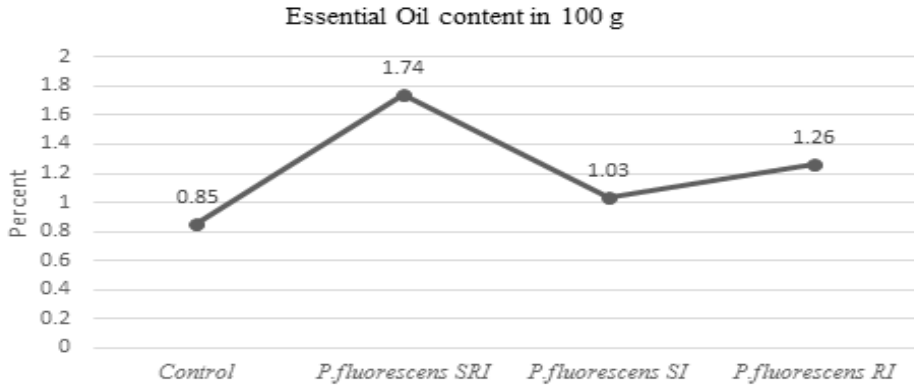

Figure 1. Effect of different inoculation methods of *P. fluorescens* on *T. kotschyanus* EO's percent. RI: Root inoculation; SI: Seed Inoculation; SRI: Seed & Root Inoculation.

first method (5 mL per seed) and the as the second method (50 mL of the bacterial suspension in contact with plant roots) was added.

Thymus growth characteristics

For plants harvested after 3 months from transplanting, the branch height, number of branches, plant dry weight, root volume, fresh and dry weight of roots were measured. After the growth period of the plants was completed, eight complete plants were removed from the middle of each cultivation line and transferred to the laboratory. After thorough washing and complete removal of sludge and dewatering with paper towels, first, the fresh weight of roots and shoots was read by a digital scale with an accuracy of 0.001 g and then by an oven at 70°C for 96 h. It was dried and the dry weight of roots and shoots was determined (Jones et al., 1993).

After irrigation, the roots were carefully removed from the culture medium and washed. A graduated cylinder with a specified volume of water was used to determine the root volume (mL). Root volume was measured by the amount of water displaced in the graduated cylinder.

Oil content

The branches were dried for 10 days in shade, then ground and oil content was estimated after the steam distillation using Clevenger's apparatus for 4 h.

Statistical analysis

Statistical analysis of the data was done by one-way (ANOVA) with Tukey's post-hoc test.

RESULTS AND DISCUSSION

In all three methods of inoculation of *P. fluorescence*, an increase in growth and the amount of essential oil was determined (Table 1). The effect of inoculation by mixed method (roots and seeds) had a greater effect on morphological traits, plants growth and percentage of thyme essential oil. The highest amount of root volume with 30 mL compared to the control (15 mL) was significant at the level of P≤0.5. The highest dry weight of the plant (24.75 g) and the highest number of branches (25), respectively, compared to the control (20 g and 18 g) showed no significant differences. The highest amount of essential oil (1.74%) was obtained in inoculation of *P. fluorescens* in the third method (seeds and roots) (Figure 1). In this study, inoculation of *P. fluorescence* by liquid suspension method with seeds, roots and rhizosphere of the plant had a positive effect on morphological characteristics and percentage of *T. kotschyanus* essential oil, which had a significant effect on some characteristics. The increased fresh and dry weight and root volume of roots in comparison with control was observed.

In general, seed and root inoculation in comparison with seed and root inoculation was significantly significant

at p <0.05 root dry weight and branch height.

The study results are in agreement with those of Eltayeb (2017), which indicated that dipping method was more effective in inoculating plants with biofertilizers, than soil application technique.

Also, the study proved that inoculating bacterial biofertilizers could increase oil content which is in an agreement with the study of Hamed et al. (2017) who used *Azotobacter chroococcum*, *Bacillus megaterium* and *Saccharomyces cerevisiae* as biofertiliers to enhance the yield of lemon grass and its essential oil content.

Conclusion

It could be concluded that the microbial inoculation methods had a great influence on the yield of *T. kotschyanus* for both the blossoms and the essential oil content. All three inoculation methods increased the growth of thyme, but combination methods had the greater effect which means that inoculation on seeds and roots can be more effective and utilizing for organic cultivation of thyme. Although the effect of inoculation of *P. fluorescens* has a positive effect on plants growth and as a biofertilizer it can be combined with arbuscular mycorrhiza fungi (AMF). But the most effective techniques or methods of inoculation of these bacteria must be considered for best results and it will be helpful for organic farming and achieving best production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Seroprevalence and behaviour at risk of zoonotic transmission of bovine brucellosis in Namentenga Province, Burkina Faso

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Bovine brucellosis is a major zoonosis caused by *Brucella abortus*. It is a disease with a high hygienic and economic impact that mainly affects breeders, handlers and consumers of dairy products and animal health professionals. It is therefore a real public health problem. The objective of this study was to assess seroprevalence and risk behaviour for zoonotic transmission of bovine brucellosis in Namentenga Province in Burkina Faso. To do this, a 2-degree random sampling was conducted. Thus, a blood sample was taken from 600 randomly selected cattle. The individual serological status of these 600 cattle in 60 farms was determined by two tests: Tamponed Antigen Test and indirect Enzyme Linked Immunosorbent Assay for confirmation. The frequency of risk behaviours towards this zoonotic disease was determined through two epidemiological questionnaires that identified known risk factors for the transmission of brucellosis between animals and humans. Individual seroprevalence was estimated at 6.8% CI 95% [5.4-8.2]. At least one animal was infected in 30% (18/60) of herds. Positivity in the indirect Enzyme Linked Immunosorbent Assay test was significantly associated with age, breed, livestock conditions and vaccination status in cattle. The most frequently observed risk behaviours in humans in Namentenga Province are assisted calving and abortions, handling of the runt without a glove, consumption of raw milk or unpasteurized curd milk and fresh cheese. In view of this result, *Brucella abortus* circulates in cattle farms in Namentenga Province. Since animal products such as milk and meat from these farms are consumed by the population, adequate measures must be taken to better protect and guide the means of prevention against this zoonosis among the inhabitants.

Key words: Bovine brucellosis, Burkina Faso, Namentenga Province, Public health, Seroprevalence, Zoonosis.

INTRODUCTION

Brucellosis is a zoonotic disease that is both a severe human disease that affects public health and an animal disease whose economic consequences are far from negligible (Calvet et al., 2010; Tialla et al., 2014; Tialla et al., 2018). Humans most often contaminate themselves through the dermal mucocutaneous pathway through contact with diseased animals and/or their products and through the digestive pathway with the consumption of infected animal products (Dao et al., 2009; Calvet et al., 2010; Tialla et al., 2014). Brucellosis slows the growth of livestock, compromises any attempt to improve animal productivity, and reduces the supply of meat to populations

(Boussini et al., 2012; Tialla, 2016). It also poses a serious threat to human health (Dao et al., 2009; Tialla, 2016). Brucellosis is the most common zoonotic infection in the world, with more than 500,000 new cases reported each year (Calvet et al., 2010). In Kyrgyzstan, brucellosis is a public health priority as the annual incidence is greater than 50 cases per 100,000 population with a seroprevalence of 8.8% in humans and 2.8% in cattle (Bonfoh et al., 2011). It posted 1,014 people in Bosnia and Herzegovina in 2008 and 458 (officially reported cases) in 2009 (Calvet et al., 2010). In Senegal, the prevalence of human brucellosis has been estimated at 60.9% among dairy cattle farmers in the peripheral area of Dakar (Tialla, 2012). Still prevalent cases among humans were found in rural Mali (23.3%) (Tasei et al., 1982), Mopti in Mali (58%) (Dao et al., 2009), Chad (2%) (Schelling et al., 2004), Ethiopia (2.6%) (Animut et al., 2009), Egypt (3%) (Afifi et al., 2005) and Tanzania (6.2%) (Kunda et al., 2007). Brucellosis can cause sterility and abortion in both animals and humans, making it a very serious problem for the health and well-being of populations (WHO, 2006; OIE, 2007; Adesokan et al., 2016). It also hinders the marketing of animals and their products (Boussini et al., 2012; Douangngeun et al., 2016; Hernandez-Mora et al., 2017). Despite recent progress in controlling this zoonotic disease, it remains common in urban, peri-urban and rural areas of developing countries (Traoré et al., 2004; Tialla et al., 2014).

Bovine brucellosis is a major zoonotic disease that can have a significant impact on public health, with transmission generally occurring through the consumption of contaminated raw milk (Dao et al., 2009; Calvet et al., 2010; Tialla et al., 2014). Females of dairy species excrete tweezers such as *Brucella melitensis*, *Brucella abortus* in their milk (Calvet et al., 2010; Makita et al., 2011). However, due to a lack of pasteurisation and cold chain, milk is often consumed in its fermented, curdled or fresh form. The consumption of raw milk appears to be a societal norm for some African populations who are convinced that in this form, milk is of good quality and cannot make them sick (Fokou et al., 2010). The consumption of raw milk and derived products is not without consequences for the health of populations. The overall objective of our study was therefore to assess seroprevalence and risk behaviour for zoonotic transmission of bovine brucellosis in the Namentenga Province of Burkina Faso.

MATERIALS AND METHODS

Study area

The study took place from 1 February 2021 to 20 July 2021 in the Namentenga Province of Burkina Faso. Located in the Centre-North region, the Namentenga Province covers an area of 6 158 km². It is under the influence of a North Sudanese climate. This climate is characterized by the alternation of two distinct types of season: a dry season from mid-November to mid-May. The dry season is subdivided into two major periods: from mid-November to the end of February, the period is relatively fresh and dry with absolute minimum temperatures of the order of 16°C. It is during this period that the cool and dry winds of North-East and South-West direction dominate widely; these are the warm continental trade winds. From March to mid-May it is the warmest period of the year with average temperatures of 40°C. Absolute highs can reach 42°C in the shade and a rainy season from mid-May to mid-November with precipitation ranging from 644.5 and 849 mm for the last 03 years. Indeed, the province is located between the isohyetes 700 and 900 mm. The average precipitation is of the order of 697.45 mm. The heaviest rains were recorded in July and August.

Study population and sampling method

The population studied consisted, on the one hand, of herds of cattle with more than ten heads (blood samples) and, on the other hand, of people in direct contact with these herds located in the Namentenga Province. The two-stage random sampling method was used (Toma et al., 2010). The first stage involved the random draw of cattle farms in our study area. As no exhaustive lists of successive sampling units were available, a preliminary survey was carried out. This survey identified 121 farms, 78 of which met the inclusion criteria. Of the 78 farms, 60 were randomly selected. The second degree involved a random draw of 10 cattle from each selected flock, or a total of 600 cattle. In each farm, two visits were carried out: the first for the awareness and written consent of each farmer for the two studies (animals and humans), and the second for collection of blood samples from animals. Two epidemiological questionnaires, one for humans and one for animals, each containing mainly closed-type questions, were developed to establish risk behaviours for this zoonotic disease. The interviews lasted an average of 20 min per person and were conducted in Mooré, Dioula or, in some cases, French. In animals, the animal health status, age, sex, breed, vaccination against brucellosis and some known symptoms of bovine brucellosis such as history of abortion and the presence of hygroma were identified. To compare young cattle with older cattle, two age classes were defined. This is Class 1 which includes animals aged 0 to 2 years and Class 2 for animals aged over 2 years. The cattle collected were divided into sex and two breed categories, the local breed and the exotic breed. The questions on farmers focused on the ethnicity, habitat and at-risk practices of the farmers surveyed, such as seasonal movements, the mode of rearing, handling of an underage without wearing a glove, assistance of the pregnant cows during stockings-low or abortions, the mode of food (consumption of raw milk and unpasteurized dairy products), and the sale and circuit of this sale.

Diagnostic methods

Blood samples were collected from the jugular vein into a dry tube identified by the farm code and the animal number. The sera were collected after centrifugation and placed in cryo-tubes using sterile disposable pipettes. For the serological diagnosis for brucellosis,

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Table 1. Individual and collective characteristics of cattle surveyed in Namentenga Province, Burkina Faso, 2021.

Variable	Cattle tested	Positive	Prevalence (%) and CI: 95%	OR and CI: 95%	p-value
Age (years)					
0-2	153	4	2.6±0.6		
>2	447	37	8.3±2.1	2.1±0.5	0.02
Total	600	41	6.8±1.4		
Sex					
Male	175	6	3.4±1.3		
Female	425	35	8.2±2.2	2.2±0.7	0.02
Total	600	41	6.8±1.4		
Breed					
Local	555	12	2.2±0.9		
Exotic	45	29	64.4±4.4	1.8±0.4	0.03
Total	600	41	6.8±1.4		
Livestock conditions					
Sedentary	106	2	1.9±0.4		
Transhumant	494	39	7.9±1.6	2.5±0.6	0.01
Total	600	41	6.8±1.4		
Vaccination status					
Vaccinated	00	00	0.0±0.0		
Unvaccinated	600	41	6.8±1.4	2.8±0.7	0.01
Total	600	41	6.8±1.4		

CI: Confidence interval; OR: odds ratio.

two serological tests were used in parallel: the Tamponed Antigen Test (TAT) and the indirect Enzyme Linked Immunosorbent Assay (iELISA). The iELISA test allows to make the confirmation. TAT is a fast, simple, cost-effective test that is considered sensitive (90%) and relatively non-specific (75%) (Mai et al., 2012). The iELISA test is considered to be very sensitive (≥95%) and very specific (≥95%) (Nielsen, 2002; Lesceu and Pourquier, 2016). The iELISA Kit (ID.vet Innovative Diagnostics) has made it possible to search for anti-*Brucella* antibodies in our serums by plate micro-method according to the recommendations of the World Organisation for Animal Health (OIE). The plates were read at 450 nm using a plate reader (Thermo SCIENTIFIC Multiskan GO Version 1.00.38). This made it possible to detect recent and old infections by highlighting IgM and IgG. The results of the analyses were interpreted according to the manufacturer's recommendations. The questionable cases were retested in order to be better determined on their serological status.

Statistical analysis

The data was entered into Epidata® and processed using Epidata Analysis® software. The variables of interest, coded in presence/absence, were positivity to the laboratory diagnostic test. The explanatory variables were individual and collective characteristics. Risk factors in cattle and risk behaviours in humans were identified using a multivariate model. A logistic regression model (proc logistic, SAS 9.3) was used to analyse positivity on the diagnostic test based on explanatory variables considered as risk factor or risk behaviour. The significance threshold was set at 5%.

Ethical consideration

This study received approval clearance from *Centre Muraz* ethical committee (number 2016-15/MS/SG/CM/IEC).

RESULTS

Individual and collective characteristics of cattle surveyed in Namentenga Province, Burkina Faso

The individual and collective characteristics of the cattle surveyed in the Namentenga Province of Burkina Faso in 2021 are recorded in Table 1.

Test positivity by iELISA was significantly associated with age, sex, breed, livestock conditions and vaccination status of cattle. These explanatory variables were considered to be identified risk factors in animals.

Table 2. Test results by iELISA and TAT tests for brucellosis in six hundred bovine serums collected in Namentenga Province, Burkina Faso, 2021.

Parameter	iELISA positive	iELISA negative	Total
TAT positive	6.3% (38/600)	0.0% (00/600)	6.3% (38/600)
TAT doubtful	0.5% (03/600)	6.5% (39/600)	7.0% (42/600)
TAT negative	0.0% (00/600)	86.7% (520/600)	86.7% (520/600)
Total	6.8% (41/600)	93.2% (559/600)	100% (600/600)

Table 3. Risk behaviours observed in humans in Namentenga Province, Burkina Faso, 2021.

Variable	OR	CI: 95%	P
Assistance in the delivery of calves	2.7	2.5-2.9	0.01
Assistance for abortions	2.3	2.1-2.5	0.02
Handling the Runt Without a glove	1.9	1.7-2.1	0.03
Consumption of unpasteurized raw milk	3.5	3.3-3.7	0.01
Consumption of unpasteurized curd milk	3.1	2.9-3.3	0.01
Consumption of fresh cheese	1.5	1.3-1.7	0.04

OR: Odds Ratio; CI: Confidence Interval.

Seroprevalence of bovine brucellosis in Namentenga Province

Of 600 serums, 38 (6.3%) and 42 (7%) gave a positive and questionable response to TAT, respectively. Following the analysis of these 80 samples using the iELISA test, the 38 TAT positive samples and 3 of the 42 TAT suspicious samples provided a positive response to the iELISA test. A total of 41 (6.8% CI 95% [5.4-8.2]) reported a positive response to the iELISA test and 30% (18/60) of the farms reported at least one positive response to the TAT and iELISA tests. The results of brucellosis testing on 600 bovine serums collected in the Namentenga Province are recorded in Table 2.

Identified risk behaviours in humans

The most common risk behaviours observed in humans are assisting with births and abortions, handling the runt without a glove, and consuming unpasteurized raw or curdled milk and fresh cheese. The results are presented in Table 3.

DISCUSSION

The individual seroprevalence of bovine brucellosis in this study was estimated at 6.8%. This result confirms the presence of brucellosis in this area of Burkina Faso, Namentenga Province. This value is almost similar to 6.6% obtained by Kubafor et al. (2000) in Ghana. It is higher than 3.61% obtained by Boussini et al. (2012) in the intra and peri-urban area of Ouagadougou in Burkina Faso. This value is also higher than those observed by some authors in Senegal (1.52%) (Kouamo et al., 2010), Chad (2.6%) (Delafosse et al., 2002), Central African Republic (3.3%) (Nakouné et al., 2004), Eritrea (5.6%) (Omer et al., 2000), Ethiopia (3.1%) (Ibrahim et al., 2010) and Zimbabwe (5.6%) (Matope et al., 2010). These differences could be related to livestock conditions. In addition, in extensive or traditional breeding, the seroprevalence of bovine brucellosis remains relatively low compared to intensive breeding (Koutinhouin et al., 2003; Kouamo et al., 2010). According to work of Terefe et al. (2017), herds reared in intensive livestock showed the highest seroprevalence. On the other hand, it should be noted that our prevalence of 6.8% is less than 13.2% obtained by Traoré et al. (2004) and 18.3% obtained by Tialla et al. (2018) in Burkina Faso, to 8.8% obtained by Sanogo et al. (2008) in Côte d'Ivoire, to 9.2% obtained by Dean et al. (2013) in Togo, 8.4% obtained by Bayemi et al. (2009) in Cameroon, 10% obtained by Arimi et al. (2005) in Kenya, 15% obtained by Bonfoh (2002) in Mali, 18.7% obtained by Chimana et al. (2010) and 23.9% obtained by Muma et al. (2007a) in Zambia, 15.8% obtained by Faye et al. (2005) and 34% obtained by Magona et al. (2009) in Uganda and 36.36% obtained by Tialla et al. (2014) in Senegal. These differences may be due to the climates, farming methods, sensitivity and specificity of the different tests used. Indeed, the hot and humid climate is favorable to the survival of the pathogen. Some breeders in our study area control the entry of sick animals into their herds by carrying out the Bengal rose test which could explain our low prevalence. Indeed, the Rose Bengale test is by far the most widely used test in sub-Saharan Africa due in particular to its simplicity, its relative good sensitivity and its low cost (Muma et al.,

2009). This test allows a rapid assessment of individual serological status at the local or regional level (OIE, 2007). However, the specificity of this test is quite low due in particular to the cross-reactions of the *Brucella* antigen with antibodies linked to other Gram-negative related bacteria such as *Yersinia enterocolitica* O:9, *Francisella tularensis*, *Vibrio cholerae*, *Escherichia coli* O:157, *Salmonella* species, and *Sternotrophomonas maltophilia* (Nielsen, 2002; Saegerman et al., 2004; Sanogo et al., 2008). This would lead to false positive serological responses that tend to overestimate the individual prevalence of brucellosis in some regions of sub-Saharan Africa (Bankole et al., 2010; Makita et al., 2011; Sanogo et al., 2012). In addition, Saegerman et al. (2004) showed that the specificity of the indirect ELISA test for the detection of brucellosis varies according to the nature of the conjugate used. The same authors reported that the specificity of the indirect ELISA test also depends on the microbism of the study area.

Our herd prevalence is well below 96.6% obtained by Tialla et al. (2014) in Senegal and 95% obtained by Tialla et al. (2018) in Burkina Faso. This could be explained by the fact that almost all the herds in these study areas were sedentary, unlike the herds in this current study which were mostly transhumant (82.3%). Indeed, according to Kouamo et al. (2010), herd prevalence remains relatively low in extensive and traditional transhumance farms. An epidemiological survey conducted by Omer et al. (2002) in Eritrea showed the influence of the livestock system, with a higher seroprevalence in dairy farming linked to higher animal density compared to a nomadic agro-pastoral system.

Test positivity was significantly associated with age, sex, breed, livestock conditions and vaccination status. Intrinsic factors such as race, sex and age can play a major role in the transmission of brucellosis. Indeed, our study found that older cattle were the most affected. The risk of infection appears to increase with age, contrary to what was described in Chad by Delafosse et al. (2002). According to Akapko and Bonarel (1987), the prevalence of brucellosis generally increases with age. This trend seems logical because with time the animal is more likely to have been infected, to remain infected and to be dangerous to other animals (Koutinhouin et al., 2003). Serological prevalence was higher in females than males. This is certainly due to the low impact of males in the epidemiology of the disease. However, our results are consistent with those of Akakpo (1987), who observed that serological prevalence in females was significantly higher than in males during the study in Burkina, Rwanda and Togo. On the other hand, this observation is reversed in Niger and appears identical for both sexes in Benin and Cameroon (Akakpo, 1987). As for the breed, the results show that exotic animals were the most affected. This could be explained by their low resistance to the harsh climatic conditions prevailing in our study area. Furthermore, the exotic breed remains particularly sensitive to pathogens (Akakpo, 1987). Extrinsic factors can also have an impact on disease transmission. Sedentary animals were the most affected. According to Delafosse et al. (2002), conditions for extensive livestock rearing limit the spread of brucellosis in contaminated herds. The mode of rearing can be considered as a risk factor for brucellosis in that contact with animals varies with the latter. Thus, in intensive farming the risk is higher, which corresponds to the findings of Akakpo (1987). No animals in this study were vaccinated against brucellosis. The antibodies detected therefore stem from contact of cattle with the pathogen. Indeed, vaccination against brucellosis is not practiced in Burkina Faso.

The most common risk behaviours observed in humans have been assisting with births and abortions, handling the runt without a glove, and consuming unpasteurized raw or curdled milk and fresh cheese. These results are consistent with those of Al-Shamahy et al. (2000) in Yemen, Dao et al. (2009) in Mali, Dean et al. (2013) in Togo and Tialla et al. (2014) in Senegal. Assisting with births and abortions and handling runts without wearing gloves are important risk factors because they are potentially very dangerous contacts. This observation was described by several authors (Bikas et al., 2003; Toma et al., 2010; Sanogo et al., 2012). The consumption of milk and non-pasteurized derived products is also a very important risk factor. This remark was also noted by Mailles and Vaillant (2007), Muma et al. (2007b) and Bonfoh et al. (2011).

Conclusion

The overall objective of this study was to assess seroprevalence and behaviour at risk for zoonotic transmission of bovine brucellosis in Namentenga Province, Burkina Faso. It was confirmed that brucellosis is present in cattle farms in the Namentenga Province in Burkina Faso, with an individual prevalence estimated at 6.8%. The most common risk behaviours observed in humans were assisting with calving and abortions, handling the runt without a glove, drinking raw milk or unpasteurized curd milk and fresh cheese. As the consumption of products from these farms is not without public health consequences, adequate measures must be taken to protect the population against this zoonosis. The implementation of an integrated approach, which takes into account the complex relationships between humans, animals and the environment within the different production systems; and the establishment of a multi-sectoral framework involving physicians, veterinarians and all public health stakeholders in the context of a one health approach should be considered.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Antibiotic resistant pattern of bacteria in untreated hospital wastewaters from Offa Local Government Area, Kwara State, Nigeria

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This study determined the prevalence and drug resistant patterns of bacteria isolated from untreated hospital wastewaters collected from selected hospitals in Offa Local Government Area of Kwara State, Nigeria. A total of 42 composite samples were aseptically collected, transported and analyzed for enumeration of microorganisms, bacteriological identification and susceptibility testing following standard procedures. The Global Positioning System (GPS) coordinates of each site location was equally taken and data obtained were analyzed using SPSS version 20. The means bacterial count population of wet season samples ranged between 7±4.00 × 10^5 and 150±43.59 × (10^5cfu/ml), while that of dry season samples ranged between 10±2.00 × 10^5 and 225±67.27 × 10^5 cfu/ml. Among the total samples, 50 bacterial isolates were detected, of which 26(52%) were from wet season samples and 24(48%) were from dry season samples. The most frequently isolated bacteria from wet season samples was *Alcaligenes faecalis* 17(65.4%) followed by *Alcaligenes aquatilis* 5(19.2%) and *Staphylococcus saprophyticus* 4(15.4%). Findings from antibiotic resistance pattern of the isolates indicated that ofloxacin (OFL) demonstrated highest antimicrobial potency against the test isolates, with Zone inhibition diameters (mm) (resistant ≤12, intermediate 13-15 and susceptible ≥ 16). Thus, hospital wastewater should be treated before discharge to prevent infectious diseases.

Key words: Hospital wastewaters, antibiotics, resistant pattern, bacteria.

INTRODUCTION

Wastewater is any water that has had its quality severely degraded by human intervention. This comprises liquid waste from private residences, businesses, industries, hospitals, and agricultural and commercial establishments (Verlicchi et al., 2010). Patient wards, surgery units laboratories, clinical wards, laundries, and other areas of a hospital generate wastewater, which has a wide range of loads based on the activities carried out (Aurelien

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et al., 2013).

Owing to several difficulties, hospital effluent has received a lot of attention in the last few years in many nations throughout the world. Hospitals are known to consume large amounts of water each day, ranging from 400 to 1200 L per day, resulting in a comparable volume of water burden (Gautam et al., 2007).

Hospital wastewater is an ideal medium for microorganisms and carries the resistant gene into the sewage system (Abdel-Rouf et al., 2012; Fekadu et al., 2015). Pharmaceuticals, radionuclides, detergent, antibiotics, antiseptic, surfactant, solvent, medicinal medication, heavy metals, and radioactive substances are among the persistent chemical compounds and complex combinations of organic matter found in hospital wastewater (Aurelien et al., 2013; Ferrando-Climent et al., 2014) plus microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli* (Anitha and Jayraaj, 2012; Ferrando-Climent et al., 2014).

Medicines are used in large quantities in hospitals on a daily basis for patient care and infection control, and a significant quantity of these antibiotics is excreted through the faeces and urine of patients, eventually reaching liquid wastes. As a result, hospital wastewater contains resistant gene and antibiotic residues, which, through selection pressure, hinders the growth of vulnerable microorganisms (Beyene and Redaie, 2011; Stalder et al., 2014). Additionally, some of these substances and bacteria defecated by patients are detected in hospital wastewater, which is then discharged into the local sewer system without being treated. As a result, this composition causes a wide range of toxicity, genotoxicity, and organic load, resulting in a completely negative influence on the natural ecosystem and an inherent health threat to humans (Wilde et al., 2013).

By functioning as a vector or reservoir of resistant gene, resistant bacteria in the environment contain transmissible gene (Pandey et al., 2011; Keen and Patrick, 2013). They are also the most hazardous microorganisms that pose a health risk to human, and wastewaters are one of the most serious contaminants that pollute the environment (Diwan et al., 2010; Pandey et al., 2011).

Antibiotic resistance is becoming a major public health issue around the world. The World Health Organization (WHO) and the European Commission (EC) have both acknowledged the necessity of researching the genesis and spread of resistance, as well as the need for control tactics (Oteo et al., 2001). In developing countries like Nigeria, improper antibiotic use, ineffective infection control programs, and a lack of better management of hospital wastewater are the main factors for antimicrobial resistance gene dissemination in the environment. Therefore, this study aimed to exploit the role of hospital wastewater as source of emerging drug resistance pathogens in the environment. The objectives of this research were to isolate bacteria associated with some hospital wastewaters and determine their antibiotics resistant patterns.

MATERIALS AND METHODS

Study area

Offa Local Government is one of the major Local Government Areas and one of the major cities of Kwara State, situated in the North Central geographical zone of Nigeria. It is situated between latitude 8° 10' 33" N, 238 km North of Lagos at longitude 4° 43' 02" E and 530 km South West of Abuja; the Federal Capital of Nigeria (ODUNA, 2020). The yearly temperature ranges between 18.5 - 37.5°C, with a humidity of between 45 – 47%, and a peak annual rainfall of 200 cm (Wikipedia, 2020). The vegetation is essentially guinea savanna, a transition zone between the Sudan savanna of the far North and the tropical rain forest vegetation of the South East. The population of Offa from the 2006 census was estimated at 88,975, while the annual growth rate was estimated at 2.3%. Kwara State where Offa is located has a population of 2.37 million from the 2006 census (PHC, 2006).

Study design and periods

This project work was a cross sectional descriptive study in which subjects were hospital wastewater samples collected from the study site between the periods of April to October, 2018 and November, 2018 to March, 2019.

Collections of hospital wastewater samples

Two sets of twenty-one (21) hospital wastewater samples were collected from Offa Local Government Area of Kwara State, Nigeria. The first set of twenty-one samples (wet season samples) were collected between the months of April – October, 2018, while the second set of twenty-one samples (dry season samples) were collected between the months of November, 2018 – March, 2019. The samples were collected from hospital laboratory units into wide mounted sterile plastic containers with screw cap tops (universal bottles) corked tightly. The containers were labeled with date, time and sites of collection, and transported inside ice packs to Microbiology Laboratory, Obakekere, FUTA for culturing of bacteria.

Isolation and enumeration of bacterial colonies from hospital wastewater samples

Five-fold serial dilution was carried out on collected hospital wastewater samples. Aliquot (1 ml) of the diluents were pipetted into Petri-dishes, pour-plated with about 20 ml of molten nutrient agar at about 45°C and allowed to gel. The isolation of bacteria from wastewater was done according to methods of Cheesbrough (2010). The emerged colonies were counted using a colony counter and values were recorded after 24 h of incubation (Marshia et al., 2016).

Preparation of pure isolates of bacterial colonies from hospital wastewater samples

A distinct colony was taken and streaked with sterile wire loop on a

freshly prepared solidified nutrient agar and incubated for 24 h at 37°C to get pure and distinct colonies. This was repeated several times until satisfactory pure isolates were obtained (Marshia et al., 2016).

Conventional identification of bacterial isolates in hospital wastewater samples

The identity of all isolates was determined using standard conventional methods as reported by Cheesbrough (2010). The bacterial isolates were cultured on nutrient agar and incubated at 37°C for 24 h and subsequently sub-cultured on to differential selective media namely Eosin Methylene blue agar and MacConkey agar. The bacterial isolates were tentatively identified by means of morphological characteristics, cellular and biochemical tests. Morphological characteristics were observed for each bacterial colony after 24 h of growth. The appearance of the colony of each isolate on the media was studied and the characteristics observed included cell shape, elevation, edge, optical characteristics, consistency colony surface and pigmentation. Biochemical tests carried out include; catalase, production of hydrogen sulphide (H_2S), indole, urease, methyl red, oxidase, coagulase, motility, citrate utilization, methyl red, voges-proskauer, starch hydrolysis and sugar fermentation. The results were compared with Bergey's Manual of Determinative Bacteriology (Fawole and Oso, 2007).

Molecular identification of bacterial isolates

Extraction of (deoxyribonucleic acid) DNA using cetyl trimethyl ammonium bromide (CTAB) method

Deoxyribonucleic acid (DNA) was extracted from hospital wastewater isolates by a standard CTAB genomic DNA isolation method as follows: 1 ml of 24 h broth culture was transferred into 1.5 ml Eppendorf tube and spun at 14,000 rpm for 30 min (to harvest the cell). 400 µl of a pre-warmed CTAB buffer (at 60°C) containing proteinase k and β-mercapto ethanol was added. Thereafter, 75 µl of 10% SDS (sodium deodycylsulphate) was added and heated in water bath at 65°C for 30 min. Five hundred micro liter (500 µl) chloroform was added, mixed for 15 min (to purify the DNA) and spun at 10,000 rpm for 10 min. The supernatant was collected in Eppendorf tube to which 500 µl isopropanol and 1 µl (100 mg/ml) RNase were added and incubated for 30 min at 37°C. The resultant mixture was kept at -20 for 24 h and spun at 10,000 rpm for 10 min. The supernatant was gently decanted and the pellet washed with 200 µl of 70% ethanol, gently mixed and spun at 10,000 rpm for 5 min. The extracted DNA was air-dried for 30 min to 1 h (to eliminate all traces of alcohol) and finally re-suspended in 200 µl of sterile distilled water (Doyle and Doyle, 1990).

Quantification of extracted deoxyribonucleic acid (DNA)

Quantification of DNA concentration and purity of the samples were measured using Nano-Drop® 2000 spectrophotometer. The ratio of 260/280 absorbance was used to assess the purity of DNA with ratios ~1.8 being accepted as pure (Akinyemi and Oyelakin, 2014).

Polymerase chain reaction (PCR) analysis of 16S

Polymerase chain reaction (PCR) analysis was run with a universal primer called 16S. The PCR mix comprises 1 µl of 10x buffer, 0.4 µl of 50 mM $MgCl_2$, 0.5 µl of 2.5 mMdNTPs, 0.05 µl of 5 units/µlTaq with 2 µl of template DNA and 6.05 µl of distilled water to make-up 10 µl reaction mix. The PCR profile used is initial denaturation temperature of 94°C for 3 min, followed by 30 cycles of 94°C for 60 s, 56°C for 60 s, and 72°C for 120 s. The final extension temperature was 72°C for 5 min and the 10°C on hold for few hours (Akinyemi and Oyelakin, 2014).

Standardization of inoculums for antibiotic sensitivity test (0.5 McFarland standard)

About 0.1 ml of 1% barium chloride was added to 9.9 ml of 1% surphuric acid which was later reconstituted into 10 ml of sterile distilled water to make 0.5 ml McFarland standard solution. The broth culture of 24 h test organism was then compared in terms of turbidity to 0.5% McFarland. A loopful of the standardized culture was used for antibiotic sensitivity assay (Andrew, 2006; Paramedics World, 2018).

Antibiotics sensitivity test

The antibiotic sensitivity of the bacterial species isolated was performed on Mueller-Hinton agar (MHA) (Merck) plates by disk diffusion method as described by the National Committee for Clinical Laboratory Standard Institute (CLSI, 2017). A 0.1 ml of each bacterial isolate was seeded into each of the Petri dishes containing Mueller-Hinton agar and allowed to stand for 30 min to enable the inoculated organisms to pre-diffuse. The commercially available discs containing the following antibiotics: ceftazidine (CAZ, 30 µg), cefuroxime (CRX, 30 µg), gentamicin (GEN, 10 µg), ceftriaxone (CTR, 30 µg), erythromycin (ERY, 5 µg) cloxacillin (CXC, 5 µg), ofloxacin (OFL, 5 µg), augmentin (AUG, 30 µg), cefixime (CXM, 5 µg), nitrofrantion (NIT, 300 µg) and ciprofloxacin (CPR, 5 µg) (Liverpool L9 7AR, UK) were aseptically placed on the surfaces of the sensitivity agar plates with a sterile forceps and incubated at 37°C overnight. Zones of inhibition after incubation were observed and the interpretation was made using susceptibility breakpoints of CLSI (2017). The diameters of the zone of inhibition around the disc were measured to the nearest millimeter using a metal caliper and the isolates were classified as sensitive, intermediate and resistant.

Data quality assurance

Sample collection, handling, transportation as well as microbiological analysis and interpretation of results were carried out following standard operating procedures (SOPs). Prior to the actual work reagents, media and antimicrobial disks were checked for expiry date, damage and storage problems. Laboratory equipment were properly cleaned and sterilized before use. Media preparation was made based on the respective manufacturer's directives. Five percent of media per batch/prepared was incubated overnight for sterility check.

Data analysis

Data obtained were analyzed using analysis of variance (ANOVA) and mean separated using Duncan's Mean Multiple Range Test (IBM-SPSS) 20 version). Differences were considered significant at $p < 0.05$.

Table 1. The samples sites coordinates and mean count of bacteria isolated from hospital wastewaters from Offa Local Government Area of Kwara State, Nigeria.

S/N	Samples' sites	Samples' sites coordinates	Wet Season	Dry Season
			Mean population x 10^5 cfu per ml	Mean population x 10^5 cfu per ml
1	A	Lat.8.15405; Long. 4.71693	150±43.59^{h}	160±10.00^{g}
2	B	Lat. 8.15445; Long. 4,72080	8±2.00^{a}	10±2.00^{a}
3	C	Lat. 8.156139; Long. 4.71465	130±43.59^{gh}	225±67.27^{i}
4	D	Lat. 8.14967; Long. 4.72209	65±5.00^{cd}	80±10.00^{bcd}
5	E	Lat. 8.16362; Long. 4.72274	9±3.00^{a}	13±3.00^{a}
6	F	Lat. 8.15417; Long. 4.71595	14±2.00^{ab}	17±1.00^{a}
7	G	Lat. 8.13615; Long. 4.71401	90±19.08^{def}	120±10.00^{f}
8	H	Lat. 8.14714; Long. 4.7092	110±18.03^{fg}	120±10.00^{f}
9	I	Lat. 8.14577; Long. 4.70525	120±10.00^{g}	202±9.17^{hi}
10	J	Lat. 8.15553; Long. 4.71627	80±10.00^{de}	110±10.00^{ef}
11	K	Lat.8.13241; Long. 4.71317	**7±4.00^{a}**	14±2.00^{a}
12	L	Lat. 8.14735; Long. 4.71178	39±8.54^{bc}	54±14.00^{bc}
13	M	Lat. 8.14655; Long. 4.72694	65±5.00^{cd}	83±3.00^{cde}
14	N	Lat. 8.15125; Long. 4.70302	71±1.00^{d}	74±3.46^{bcd}
15	O	Lat. 8.14961; Long. 4.71268	103±13.00^{efg}	115±5.00^{f}
16	P	Lat. 8.14838; Long. 4.72694	110±10.00^{fg}	120±10.00^{f}
17	Q	Lat. 8.15884; Long. 4.72436	16±6.00^{ab}	11±1.00^{a}
18	R	Lat. 8.16861; Long. 4.71516	32±3.00^{ab}	51±2.00^{b}
19	S	Lat. 8.15529; Long. 4.71584	69±8.54^{d}	89±16.52^{def}
20	T	Lat. 8.16534; Long. 4.71088	30±5.00^{ab}	20±4.58^{a}
21	U	Lat. 8.15347; Long. 4.71563	16±3.46^{ab}	190±10.00^{h}

Values are mean ±SD of replicates (n=3). Values with the same alphabet in the same column are not significantly different while values with different alphabet are significantly different ($\alpha < 0.05$). S/N = serial numbers, A-U = samples collection sites, Lat=latitude, Log=longitude.

RESULTS

Mean count of bacteria isolated from hospital wastewaters

Generally, there were significant differences ($p<0.05$) in means count of bacteria in all wastewaters samples. Samples from Site C had the highest bacterial count (225 ±67.27 x 10^5 cfu per ml) during the dry season. On the other hand, during the wet season, Site A had the highest (150 ±43.59 x 10^5 cfu per ml) and Site K had the lowest bacterial count (7 ±4.00 x 10^5 cfu per ml) during the wet season (Table 1).

Bacteria isolated from hospital wastewaters

Alcaligenes faecalis was found present almost in the entire forty-two (42) sample sites (both during the dry and wet season periods), except in sites O and P (during the dry season), and sites J, O, Q and R (during the wet season). *Staphylococcus saprophyticus* were found present only in sites O and P (during the dry season), and also in sites O, Q and R (during the wet season). However, *Alcaligenes aquatilis* was found alone in site J during wet season (Table 2).

Prevalence/Percentage of bacterial isolates

A. faecalis made up to 19(79.2%) of the total bacteria isolated during the dry season and 17(65.4%) during the wet season while *A. faecalis* strain JF3 is more prevalent with percentage occurrence 26.9% during the wet season and strain G68 and KWW84 with percentage occurrence of 29.2 during the dry season (Table 3).

Antibiotics resistance pattern of bacterial isolates from hospital wastewater

The *in-vitro* antibiotic resistance pattern of the bacteria isolated from hospital wastewater indicated that all bacteria isolated were susceptible to ofloxacin during the dry and wet season periods, but were resistant to ceftazidine, cefuroxime, erythromycin, cloxacillin,

Table 2. Bacterial isolated from hospital wastewater sample collected from Offa Local Government Area Kwara State, Nigeria.

S/N	Samples' sites	Wet season microorganisms	Dry season microorganisms
1	A	*A. faecalis* (JF3)OFW_1 *A. aquatilis* (YFMCD4.2)OFW_1	*A. faecalis* (G68)OFD_1
2	B	*A. faecalis* (JF3)OFW_2	*A. faecalis* (G68)OFD_2
3	C	*A. faecalis* (JF3)OFW_3 *A. aquatilis* (YFMCD4.2)OFW_2	*A. faecalis*(G68)OFD_3
4	D	*A. faecalis* (JF3)OFW_4	*A. faecalis*(Z1116)OFD_1
		S. saprophyticus (FELA049)OFW_1	
5	E	*A. faecalis* (M453B1)OFW_1	*A. faecalis*(G68)OFD_4
6	F	*A. faecalis* (ISJ128)OFW_1 *A. aquatilis* (YFMCD4.2)OFW_3	*A. faecalis*(Z1116)OFD_2
7	G	*A. faecalis*(JF3)OFW_5	*A. faecalis*(G68)OFD_5
8	H	*A. faecalis*(ISJ128)OFW_2	*A. faecalis*(KWW84)OFD_1
9	I	*A. faecalis*(JF3)OFW_6	*A. faecalis*(KWW84)OFD_2
10	J	*A. aquatilis* (YFMCD4.2)OFW_4	*A. faecalis*(G68)OFD_6
11	K	*A. faecalis*(JF3)OFW_7 *A. aquatilis* (YFMCD4.2)OFW5	*A. faecalis*(Z1116)OFD_3
12	L	*A. faecalis* (M453B1)OFW_2	*A. faecalis*(G68)OFD_7 *S. saprophyticus* (FELA049)OFD1
13	M	*A. faecalis* (M453B1)OFW_3	*A. faecalis*(KWW84)OFD_3
14	N	*A. faecalis* (M453B1)OFW_4	*A. faecalis*(KWW84)OFD_4
15	O	*S. saprophyticus* (FELA049)OFW_2	*S. saprophyticus* (FELA049)OFD_2
16	P	*A. faecalis*(ISJ128)OFW_3	*S. saprophyticus* (FELA049)OFD_3
17	Q	*S. saprophyticus* (FELA049)OFW_3	*A. faecalis*(Z1116)OFD_4
18	R	*S. saprophyticus* (FELA049)OFW_4	*A. faecalis*(KWW84)OFD_5
19	S	*A. faecalis* (M453B1)OFW_5	*A. faecalis*(Z1116)OFD_5 *S. saprophyticus* (FELA049)OD_4
20	T	*A. faecalis*(ISJ128)OFW_4	*A. faecalis* (KWW84)OFD_6
21	U	*A. faecalis*(ISJ128)OFW_5	*A. faecalis* (KWW84)OFD_7 *S. saprophyticus* (FELA049)OFD5

A. aquatilis = Alcaligenes aquatilis, A. faecalis =Alcaligenes faecalis, S. saprophyticus = Staphylococcus saprophyticus. OFD = Offa Dry season isolate, OFW = Offa Wet season isolate, S/N = serial number, A-U = samples sites locations.

Table 3. Prevalence/percentage of bacteria present in hospital wastewaters collected from Offa Local Government Area of Kwara State, Nigeria.

Bacterial isolates (wet season)	N (%)	Bacterial isolates (dry season)	N (%)	Total (%)
A. faecalis 7(JF3)	7(26.92)	-	0(0.00)	7(14.00)
-	0(0.00)	*A. faecalis* 7(G68)	7(29.17)	7(14.00)
A. faecalis 5(M4S3B1)	5(19.23)	-	0(0.00)	5(10.00)
-	0(0.00)	*A. faecalis* 5(Z1116)	5(20.83)	5(10.00)
A. faecalis 5(ISJ128)	5(19.23)	-	0(0.00)	5(10.00)
-	0(0.00)	*A. faecalis* 7(KWW84)	7(29.17)	7(14.00)
A. aquatilis 2(YFMCD4)	5(19.23)	-	0(0.00)	5(10.00)
*S. saprophyticus*4(FELA049)	4(15.39)	*S. saprophyticus* 5(FELA049)	5(20.83)	9(18.00)
Total	26(100-00)		24(100.00)	50(100.00)

N = number of isolates, % = percentage present, - = absent.

Table 4. Percentage susceptible, intermediate and resistance of antibiotics on bacterial isolates of hospital wastewater sample collected from Offa Local Government Area, Kwara State, Nigeria during wet season.

S/N	Antibiotics	No. of tested isolates	No. of susceptible isolates	No. of intermediate isolates	No. of resistant isolates	% Susceptibility	% intermediary	% Resistance
1	Ceftazidine	26	0	0	26	0	0	100
2	Cefuroxime	26	0	0	26	0	0	100
3	Gentamicin	26	16	5	5	61.5	19.2	19.2
4	Ceftriaxone	26	4	0	22	15.4	0	84.6
5	Erythromycin	26	0	5	21	0	19.2	80.8
6	Cloxacillin	26	0	0	26	0	0	100
7	Ofloxacin	26	26	0	0	100	0	0.0
8	Augmentin	26	0	0	26	0	0	100
9	Cefixime	26	0	0	26	0	0	100
10	Nitrofurantoin	26	10	0	16	38.5	0	61.5
11	Ciprofloxacin	26	10	16	0	38.5	61.5	0.0

Table 5. Percentage susceptibility, intermediary and resistance of antibiotics on bacterial isolates of hospital wastewater samples collected from Offa Local Government Area, Kwara State, Nigeria during dry season.

S/N	Antibiotics	No. of tested isolates	No. of susceptible isolates	No. of intermediate isolates	No. of resistant isolates	% Susceptibility	% intermediary	% Resistance
1	Ceftazidine	24	0	0	24	0	0	100
2	Cefuroxime	24	0	0	24	0	0	100
3	Gentamicin	24	17	7	0	70.8	29.2	0
4	Ceftriaxone	24	0	12	12	0	50	50
5	Erythromycin	24	0	5	19	0	20.8	79.2
6	Cloxacillin	24	0	0	24	0	0	100
7	Ofloxacin	24	24	0	0	100	0	0
8	Augmentin	24	0	0	24	0	0	100
9	Cefixime	24	0	0	24	0	0	100
10	Nitrofurantoin	24	5	0	19	20.8	0	79.2
11	Ciprofloxacin	24	5	14	5	20.8	58.3	20.8

augmentin and cefixime (Tables 4 and 5).

Antibiotics sensitivity profile of bacterial isolates from hospital wastewater

All bacterial isolates of hospital wastewater collected both during the wet and dry season periods were found to be 100% resistant to ceftazidine, cefuroxime, cloxacillin, augmentin and cefixime. The isolates were equally found to be 100% sensitive to ofloxacin. *A. faecalis* strain JF3, *A. faecalis* strain ISJI28 and *S. saprophyticus* strain FELA049 were found to be 100% sensitive to gentamicin, while only *A. faecalis* strain M4S3B1 was 100% resistant to gentamicin. Also, among the isolates, only *A. faecalis* strain M4S3B1 and Z1116 were 100% sensitive to nitrofurantoin and ciprofloxacin (Tables 6 and 7).

Total antibiotics sensitivity profile of bacterial isolates of hospital wastewater samples

The total resistance of bacterial isolates from hospital wastewater collected during the wet season was higher for ceftazidine, cefuroxime, cloxacillin, augmentin and cefixime 26/26 (100%) followed by ceftriaxone 22/26 (84.6%), erythromycin 21/26 (80.8%) and nitrofurantoin 16/26 (61.4%). However, relatively lower resistances were observed among bacterial isolates to gentamycin 5/26 (19.2%), ofloxacin 0/26 (0%) and ciprofloxacin 0/26 (0%).

Table 6. Antibiotic sensitivity profile of hospital wastewater isolates of sample collected from Offa Local Government Area, Kwara State, Nigeria during wet season.

Bacterial Isolate		Antibiotics Used N (%)										
		CA Z	CRX	GEN	CTR	ERY	CXC	OFL	AUG	CXM	NIT	CPR
Alikaligenes faecalis JF3(7)	S	0(0)	0(0)	7(100)	0(0)	0(0)	0(0)	7(100)	0(0)	0(0)	0(0)	0(0)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	7(100)
	R	7(100)	7(100)	0(0)	7(100)	7(100)	7(100)	0(0)	7(100)	7(100)	7(100)	0(0)
A. aquatilis YFMCD(5)	S	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	5(100)	0(0)	0(0)	0(0)	0(0)
	I	0(0)	0(0)	5(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	5(100)
	R	5(100)	5(100)	0(0)	5(100)	5(100)	5(100)	0(0)	5(100)	5(100)	5(100)	0(0)
Staphylococcus saprophyticus FELA049(4)	S	0(0)	0(0)	4(100)	4(100)	0(0)	0(0)	4(100)	0(0)	0(0)	0(0)	0(0)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	4(100)
	R	4(100)	4(100)	0(0)	0(0)	4(100)	4(100)	0(0)	4(100)	4(100)	4(100)	0(0)
A. faecalis M4S3B1(5)	S	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	5(100)	0(0)	0(0)	5(100)	5(100)
	I	0(0)	0(0)	0(0)	0(0)	5(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	R	5(100)	5(100)	5(100)	5(100)	0(0)	5(100)	0(0)	5(100)	5(100)	0(0)	0(0)
A. faecalis ISJ128(5)	S	0(0)	0(0)	5(100)	0(0)	0(0)	0(0)	5(100)	0(0)	0(0)	5(100)	5(100)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	R	5(100)	5(100)	0(0)	5(100)	5(100)	5(100)	0(0)	5(100)	5(100)	0(0)	0(0)

Alcaligenes faecalis strain JF3, *A. aquatilis* strain YFMCD4, *Staphylococcus saprophyticus* strain FELA049, *A. faecalis* strain M4S3B1, *A. faecalis* strain ISJ128 Offa R = Resistant, S= Susceptible, I = intermediate, ceftazidine (CAZ, 30 µg), cefuroxime (CRX, 30 µg), gentamicin (GEN, 10 µg), ceftriaxone (CTR, 30 µg), erythromycin (ERY, 5 µg) cloxacillin (CXC, 5 µg), ofloxacin (OFL, 5 µg), augmentin (AUG, 30 µg), cefixime (CXM, 5 µg), nitrofurantoin (NIT, 300 µg), ciprofloxacin (CPR, 5 µg).

Table 7. Antibiotic sensitivity profile of isolates of hospital wastewater sample collected from Offa Local Government Area, Kwara State, Nigeria during dry season during.

Bacterial isolate		Antibiotics used N (%)										
		CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	CXM	NIT	CPR
Alikaligenes faecalis G68(7)	S	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	7(100)	0(0)	0(0)	0(0)	0(0)
	I	0(0)	0(0)	7(100)	7(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	7(100)
	R	7(100)	7(100)	0(0)	0(0)	7(100)	7(100)	0(0)	7(100)	7(100)	7(100)	0(0)
A. faecalis Z1116(5)	S	0(0)	0(0)	5(100)	0(0)	0(0)	0(0)	5(100)	0(0)	0(0)	5(100)	5(100)
	I	0(0)	0(0)	0(0)	0(0)	5(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	R	5(100)	5(100)	0(0)	5(100)	0(0)	5(100)	0(0)	5(100)	5(100)	0(0)	0(0)
A. faecalis KWW84(7)	S	0(0)	0(0)	7(100)	0(0)	0(0)	0(0)	7(100)	0(0)	0(0)	0(0)	0(0)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	7(100)
	R	7(100)	7(100)	0(0)	7(100)	7(100)	7(100)	0(0)	7(100)	7(100)	7(100)	0(0)
Staphylococcus *Saprophyticus* FELA049(5)	S	0(0)	0(0)	5(100)	0(0)	0(0)	0(0)	5(100)	0(0)	0(0)	0(0)	0(0)
	I	0(0)	0(0)	0(0)	5(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	R	5(100)	5(100)	0(0)	0(0)	5(100)	5(100)	0(0)	5(100)	5(100)	5(100)	5(100)

Alcaligenes faecalis strain G68, *A. faecalis* strain Z1116, *A. faecalis* strain KWW84, *Staphylococcus saprophyticus* strain FELA049, R = Resistant, S= Susceptible, I = Intermediate, ceftazidine (CAZ, 30 µg), cefuroxime (CRX, 30 µg), gentamicin (GEN, 10 µg), ceftriaxone (CTR, 30 µg), erythromycin (ERY, 5 µg) cloxacillin (CXC, 5 µg), ofloxacin (OFL, 5 µg), augmentin (AUG, 30 µg), cefixime (CXM, 5 µg), nitrofurantoin (NIT, 300 µg), ciprofloxacin (CPR, 5 µg).

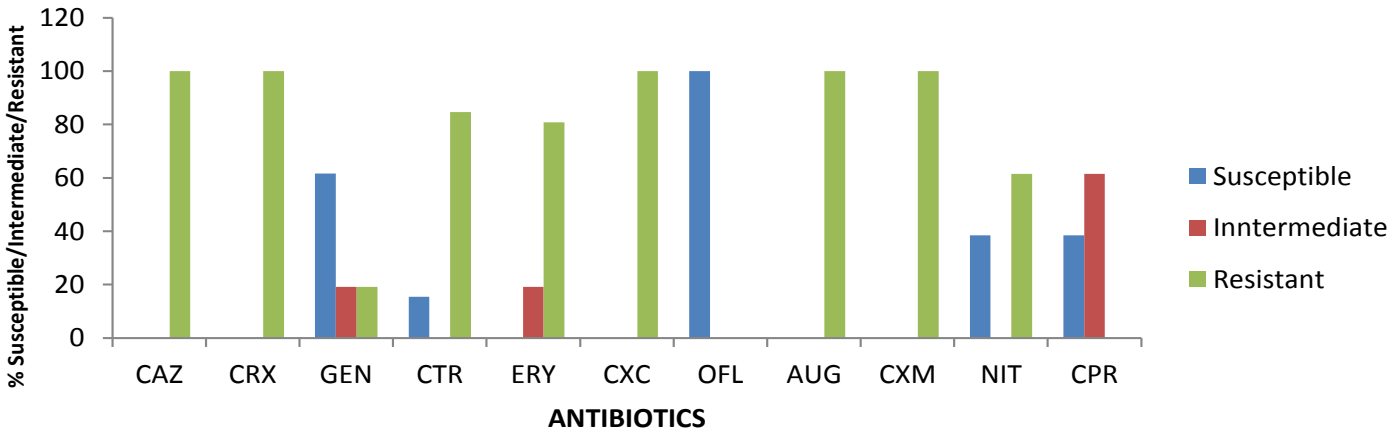

Figure 1. Total antibiotic sensitivity profile of bacterial isolates of hospital wastewater samples collected from Offa Local Government Area, Kwara State, Nigeria during wet season. Ceftazidine (CAZ, 30 µg), cefuroxime (CRX, 30 µg), gentamicin (GEN, 10 µg), ceftriaxone (CTR, 30 µg), erythromycin (ERY, 5 µg) cloxacillin (CXC, 5 µg), ofloxacin (OFL, 5 µg), augmentin (AUG, 30 µg), cefixime (CXM, 5 µg), nitrofurantoin (NIT, 300 µg), ciprofloxacin (CPR, 5 µg).

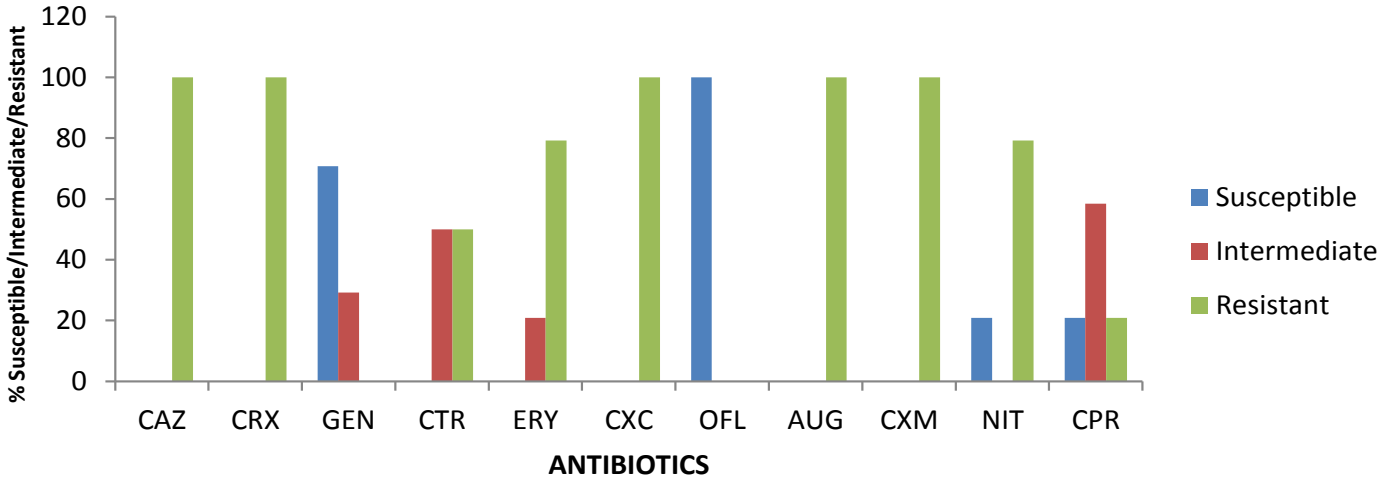

Figure 2. Total antibiotic sensitivity profile of bacterial isolates of hospital wastewater sample collected from Offa Local Government Area, Kwara State, Nigeria during dry season. Ceftazidine (CAZ, 30 µg), cefuroxime (CRX, 30 µg), gentamicin (GEN, 10 µg), ceftriaxone (CTR, 30 µg), erythromycin (ERY, 5 µg) cloxacillin (CXC, 5 µg), ofloxacin (OFL, 5 µg), augmentin (AUG, 30 µg), cefixime (CXM, 5 µg), nitrofurantoin (NIT, 300 µg), ciprofloxacin (CPR, 5 µg).

Similarly, higher resistance was recorded during dry season for ceftazidine, cefuroxime, cloxacillin, augmentin and cefixime 24/24 (100%) followed by erythromycin ciprofloxacin 5/24 (20.8%) (Figures 1 and 2).

DISCUSSION

The values of bacterial plate counts recorded during the dry and wet season periods in this research exceeded the permissible limit of Environment Protection Agency, EPA (2002) and Health Protection Agency, HPA (2005) 19/24 (79.2%), nitrofurantoin 19/24 (79.2%) and ceftriaxone 12/24 (50%). However, relatively lower resistance was observed among bacterial isolates to (<1000 cfu/ml) and also failed to fulfill the requirements of the revised guidelines on the quality of treated wastewater used in agriculture, in public parks (<5 × 10^3cfu/100 ml) (Carr et al., 2004). High density of bacteria recorded both during wet and dry season periods, was an indication of environmental pollution due to human activities. This finding agrees with results recorded by Tsegahun et al. (2017) on wastewater at Ayder Referral Hospital, Mekelle North Ethiopia. Also, there were

significant differences ($p<0.05$) in the means count of bacteria in all wastewater samples analyzed.

The variations observed in the values of the mean bacterial populations among hospitals in Offa Local Government Area may be due to variation in the rate of people's patronage at different hospitals which is dependent on location, accessibility, health care facility and personnel available. Also, it was observed that higher density of microbial population was obtained during dry season than wet season. Therefore, preference of specific microorganisms to specific temperature ranges for growth and activity can have impacts on the composition of the microbial community (Fierer and Schimel, 2003; Singh et al*.,* 2010).

The bacterial isolates found present in this study were different from that obtained by Tsegahun et al. (2017) on wastewater from Ayder Referral Hospital, Mekelle North Ethiopia, where *Klebsiella* spp, *Pseudomonas aeruginosa*, *S. aureus*, *E. coli* and *Salmonella* spp. were detected. The findings in the study of Tsegahun et al. (2017) also disagrees with the observation of Fekadu et al*.* (2015) who reported presence of *Salmonella* spp., *Shigella* spp., *E. coli* and *S. aureus* from effluent collected from Hawassa University Referral Hospital, Ethiopia. Also, isolates in this study were different from the study in India by Chitnis et al*.* (2000) that showed large numbers of enteric-bacteria *S. aureus* and *P. aeruginosa*. It is also dissimilar to work of Danchaivijitr et al*.* (2005) and Salem et al. (2011) who claimed availability of pathogenic bacteria like *Vibrio* spp. and *Salmonella* spp. in Thailand and Tunisia hospital effluents respectively. However, the findings of this study agreed with the work of Tsegahun et al. (2017) that confirmed the presence of *S. aureus* and CoNS (coagulase negative *Staphylococcus*) in treated hospital wastewater collected from Ayder Referral Hospital, Mekelle North, Ethiopia. The absence of some pathogenic bacteria in the hospital wastewater analyzed may be due to variation in geographical and climatically condition as shifting of microbial community occurs in favour of the species which are better adapted to higher temperatures and have accelerated rates of growth (Castro et al*.,* 2010; Singh et al., 2010; Fierer and Schimel, 2003). More so, inter-specific competition among microorganisms may cause shift in microbial community, such that microorganisms that compete favorably among the mixed community due to several factors such as population density, inhibitory metabolites, and so on will be prevailing. The highest prevalence of *A. faecalis* may be due to the fact that it is highly associated with urinary tract infection (UTI) which is common in hospital environment, and production of toxic exudates might also favour survival of *Staphylococcus* spp.

The resistance of all the bacterial isolates to ceftazidine, cefuroxime, cloxacillin, augmentin and cefixime was similar to finding of Katouli et al*.* (2012) where isolates showed simultaneous resistance for ampicillin with clavulanic acid, cotrimoxazole, tetracycline, first, second and third generation cephalosporins in the final effluent of wastewater treatment plant in India. Study in Alexandria, Egypt also showed the presence of antibiotic resistant extended spectrum beta-lactamase (ESBL) producing bacteria at the end of wastewater purification process (Amine, 2013), posing a risk of its spread to the environment and subsequent human and animal exposure. Overall resistance of bacteria isolated during the wet season for ceftriaxone, erythromycin, nitrofurantoin and gentamycin were found to be 84.6, 80.8, 61.4 and 19.2% respectively. Similarly, all bacterial isolates from hospital wastewater collected during the dry season were found to be 100% resistant to ceftazidine, cefuroxime, cloxacillin, augmentin and cefixime, and were equally found to be 100% sensitive to ofloxacin. Similar observation was reported by Iweriebor et al. (2015) from Alice, Eastern Cape province of South Africa and European countries. Also, Servais and Passerat (2009) confirmed higher rate of resistance in bacterial isolates from final effluent of wastewater treatment plant. The resistance of microbes to these antibiotics may be due to abuse of these antibiotics by their users or their failure to adhere to instruction given by the physicians (Davey et al*.,* 2002). More so, presence of high percentage of drug resistant isolates from hospital wastewater suggests that, hospital wastewater could have contributed massively to the resistances observed among the isolates from the final effluent. These can be due to the fact that, hospital wastewater contains a diverse group of pathogenic commensals and environmental bacteria. This characteristic composition makes sewage particularly suitable ecological niche for the growth and spread of antibiotic resistance due to selection pressure and horizontal gene transfer (Davies and Davies, 2010; Periasamy and Sundaram, 2013; Cantona et al*.*, 2013).

CONCLUSION AND RECOMMENDATIONS

A. faecalis was the most predominant among the isolates from hospital wastewater samples analysed followed by *S. saprophyticus*, which exceeded the WHO, HPA, EPA and FAO standard permissible levels. The high prevalence of drug resistant isolates from hospital wastewater samples analyzed suggests their persistence in the hospital environment, and their ability to spread antibiotic resistance due to selection pressure and horizontal gene transfer. Therefore, patients are advised to adhere strictly to the directives of the physician in administration of drugs so as to reduce the cases of antibiotics resistance. Also, adequate liquid waste treatment system should be developed to disinfect pathogens in hospital wastewater effluent before discharging into municipal water supply, so as to prevent diseases associated with hospital wastewater effluent microbes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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