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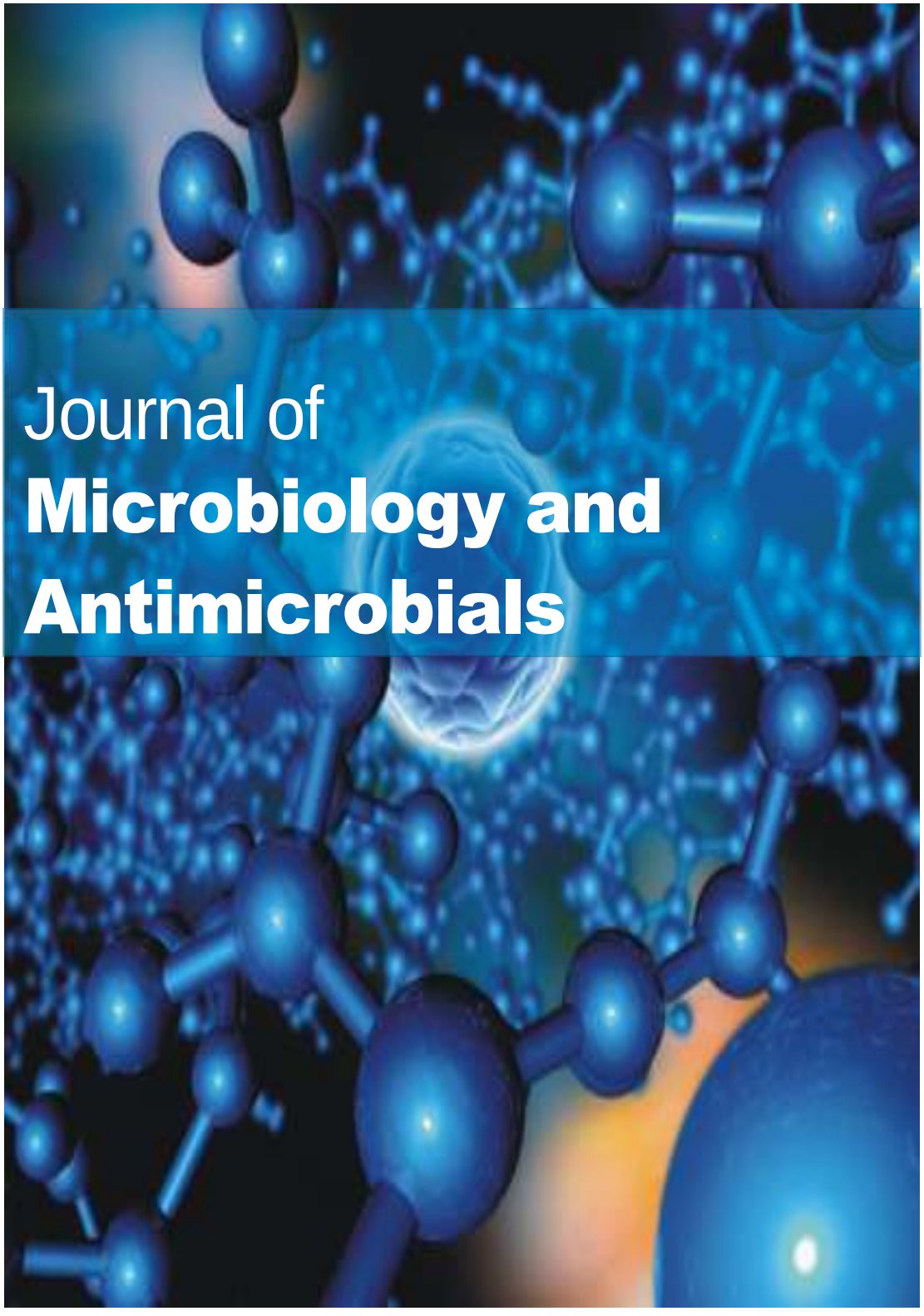

Journal of Microbiology and Antimicrobials

January-June 2021
ISSN 2141-2308
DOI: 10.5897/JMA

ACADEMIC JOURNALS

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Table of Content

Vol. 13(1), pp. 1-10, January-June 2021
DOI: 10.5897/JMA2020.433
Article Number: 46993DD65975
ISSN: 2141-2308

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Journal of Microbiology and Antimicrobials

Full Length Research Paper

Prevalence of integrons in Enterobacteriaceae obtained from clinical samples

Joy Ndidiamaka Barns[1], Cajethan Onyebuchi Ezeamagu[1*], Munachimso Esther Nkemjika[1] and Tolulope Sherifat Akindele[2]

[1]Department of Microbiology, Babcock University, Ilishan-Remo, Ogun State, Nigeria.
[2]Department of Laboratory Technology, Abraham Adesanya Polytechnic, Ijebu-Igbo, Ogun State, Nigeria.

Received 5 June, 2020; Accepted 15 January, 2021

Multi-drug resistant bacteria are a public health problem associated with high morbidity and mortality globally. This increasing drug resistance has been linked to gene exchange between bacteria. Integrons are gene exchange systems and are known to play a significant role in the acquisition and dissemination of antimicrobial resistance genes especially in Gram negative bacteria. Hence, this study aims to evaluate integrons in members of Enterobacteriaceae obtained from clinical samples. Forty-nine (49) isolates identified as *Escherichia coli* (45), *Proteus mirabilis* (2), *Shimwellia blattae* (1), and *Klebsiella pneumoniae* (1) were resistant to amoxicillin/clavulanate, cefuroxime, cefixime and ceftazidime while 43(87.76%), 45(91.84%), 46(93.88%) and 29(59.18%) of these strains were resistant to gentamicin, ofloxacin, ciprofloxacin and nitrofurantoin, respectively. Class 1 integrons were found in *E. coli* (18), *Klebsiella pneumoniae* (1) and *Proteus mirabilis* (1). This study revealed that large proportion of the strains studied were multi-drug resistant, and possessed integrons. Consequently, there is a need for proactive antibiotic surveillance system in both healthcare and community settings with a view to reducing the incidence and spread of antibiotic resistance genes between different species of bacteria.

Key words: Enterobacteriaceae, clinical samples integrons, multidrug resistance.

INTRODUCTION

Enterobacteriaceae is a large family of Gram-negative bacteria with rod shape, non-spore forming and capable of fermenting arrays of carbohydrates (Octavia et al., 2014). Clinical and community associated infections in humans have been caused by this group of bacteria especially *Klebsiella, Proteus*, *Citrobacter, Serratia*, *Escherichia, Enterobacter, Yersinia, Salmonella* and *Shigella* with 4.5 billion cases annually and 1.9 million deaths (Jarzab et al., 2011; Ye et al., 2018). Infections caused by this group of bacteria are preferably treated with broad beta-lactam antibiotics like carbapenems and cephalosporins (Khyade et al., 2018).

Currently, multi-drug resistant bacteria have become an increasing issue in healthcare system due to their ever increasing morbidity and mortality globally (Ye et al., 2018; Stephen and Kennedy, 2018; Nabti et al., 2019). Increasing drug resistance in Enterobacteriaceae has been a problem in clinical and community environments

*Corresponding author. E-mail: onyezecajeth@yayoo.com. Tel: +2348033661588.

as a result of its attendant consequences. Arrays of different mechanisms have led to a spread of resistance genes in bacteria usually via horizontal gene transfer. This global dissemination and widespread of resistance genes among bacteria can threaten the therapeutic management of patients in the event of infections, thus, endangering the effectiveness of last resort antibiotics available. Resistance occurs intrinsically with time, but can be amplified quickly as a result of selective pressure ignited by inappropriate use or rather abuse of antibiotics (Morosini, 2017).

Microbial drug resistance will continue to be on the increase unless strict stewardship programs are established. Their burdens include prolonged hospitalization, recurrent infections, economic cost, and high mortality rate (Alemayehu et al., 2019).

The increasing drug resistance has been linked to gene exchange between bacteria occasioned by antibiotic pressure resulting from an excessive and unregulated use of these agents in various human applications (Ye et al., 2018). Multi-drug resistance although frequently acquired from healthcare settings can spread via chromosomal mutations and disseminated by extra chromosomal associated elements (such as plasmids, transposons, and integrons) acquired from other bacteria (Rezaee et al., 2011). Integrons are gene exchange systems and are known to play a significant role in the acquisition and dissemination of antimicrobial resistance genes especially in Gram negative bacteria (Domingues et al., 2012). Also, integrons are composed of integrase gene, the promoter and the attachment site (Rezaee et al., 2011). They are responsible for the integration and dissemination of resistance genes among the bacteria. Several classes of integrons have been described based on the amino acid sequences of respective integrase genes, but class 1 and 2 integrons are the most prevalent in MDR gram-negative bacteria which is associated with antibiotic treatment failure (Domingues et al., 2012; Deng et al., 2015; Hadi et al., 2018).

In Nigeria where the antibiotic surveillance system is at its infancy, and abuse of antibiotics eminent, there is therefore an urgent need to evaluate the extent of prevalence of this resistance determinant (integrons) in Enterobacteriaceae obtained from clinical setting as only pocket of reports in *Escherichia coli* and *Pseudomonas aeruginosa* had been investigated (Chah et al., 2010; Odumosu et al., 2013; Igbinosa and Obuekwe (2014); Adesoji et al., 2017; Odetoyin et al., 2018). Hence, this study was carried out to detect the prevalence of integrons in Enterobacteriaceae obtained from clinical samples.

MATERIALS AND METHODS

Sample collection

Three hundred and fifty-nine clinical samples (urine 104, stool 87, endocervical swab 86 and high vaginal swab 83) of patients submitted to Microbiology laboratory for normal routine services were collected within a six-month period (January to June 2019). The samples for microbiological analysis were transferred aseptically into a transport medium (Buffered peptone water, Oxoid LTD, Basingstoke, Hamshire, England) and transported to the Microbiology laboratory, for analysis. Samples were processed microbiologically within 48 h of collection on MacConkey and Eosin Methylene blue agar plates (EMB) (Biomark Laboratories, India) and incubated at 37°C. After 24 h, suspected *E. coli* strains showing green metallic sheen were purified and sub-cultured onto MacConkey sorbitol agar (MSA) (Biomark Laboratories, India) petri plates for the presumptive identification of *E. coli* 0157:H7. Colonies on MacConkey agar were also purified and stored in 40% glycerol at -20°C (Oladipo and Fajemilo, 2012; Moghaddam et al., 2015). Ethical clearance was authorized (BUHREC543/19).

DNA extraction

Qick-DNA™ miniprep plus kit (Zymo research, Biolab, USA) was used for the extraction. Briefly, physiological young culture samples of Enterobacteriaceae (200 µl) were added to micro tubes. An equal volume of biofluid cell buffer (Red) was added with the addition of 20 µl Proteinase K.

The contents contained in the tubes were thoroughly vortex for 10-15 s and then incubated at 55°C for 10 min. A volume of Genomic Binding Buffer (420 µl) was added to the digested samples and thoroughly vortex for 10-15 s. The mixtures were transferred to a Zymo-Spin™ IIC-XLR Column in collection tubes and centrifuged at ≥ 12,000 r.p.m. The collection tubes with the flow through were discarded. DNA Pre-Wash Buffer of 400 µl was added to the spin columns in a new collection tubes and centrifuged at ≥ 12,000 r.p.m. Exactly 700 µl g-DNA Wash Buffer was added to the spin columns and centrifuged at ≥ 12,000 r.p.m.

The collection tubes were discarded. g-DNA wash buffer of 200 µl was added to the spin columns and Centrifuge at ≥ 12,000 r.p.m. The collection tubes with the flow were discarded. The spin columns were transferred to a clean micro tube and exactly 50 µl of DNA elution buffer was added directly on the matrix. It was incubated for 5 min at room temperature, then centrifuged at maximum speed for 1 min to elute the DNA. The eluted DNA was used immediately for molecular-based applications.

Polymerase chain reaction (PCR) detection of *E. coli* and its shiga toxins by polymerase chain reaction

All isolates suspected of *E. coli* based on phenotypic screening were identified using specific primers targeting the *uid* gene and screened for O157:H7 strains. PCR mixture (25 µl) contained 12.5 µl solution of the master mix (New England Biolabs), 9.5 µl H_20, 0.5 µl 10 mM of each *uid* primers and 2.0 µl of DNA template. Amplification was carried out using miniPCR (USA) with the following thermal cycling profile: initial denaturation at 94°C for 3 min, denaturation at 94°C for 30s, annealing as indicated in Table 1 for 30s and extension at 68°C for 30s and a final extension at 68°C for 5 min with period of 30 cycles. Amplicons were analysed by agarose gel electrophoresis.

Species barcoding

Seven representatives of isolates were selected for sequencing. Genomic DNA extracted above was quantified by NanoDROP 3300 spectrometer (Thermo Fisher Scientific Inc., USA). The quality of DNA was verified by 1.5 agarose gel electrophoresis prior to the PCR amplification reaction. The 16S rRNA of the bacteria was amplified using PCR with primers 341F 5'-CCTACGGGAGGCAGCAG3' and R806:5'GGACTACHVGGGTWTCTAAT-3'as described above. The

Table 1. Primers used for amplification of the integrase gene and its variable regions.

Primers	Sequence; 5'-3'	Genes	Amplicon size (bp)	Tm (°C)	References
hep35F	TGCGGGTYAARGATBTKGATTT	Int1,2,3	491	37	White et al. (2001)
hep36R	CARCACATGCGTRTARAT				
hep58F	TCATGGCTTGTTATGACTGT	Int1	Variable	46	White et al. (2001)
hep59R	GTAGGGCTTATTATGCACGC				
stx1F	ATAAATCGCCATTCGTTGACTAC	Stx1	180	51	Paton and Paton (1998)
stx1R	AGAACGCCCACTGAGATCATC				
stx2F	GGCACTGTCTGAAACTGCTCC	Stx2	255	52	Paton and Paton (1998)
stx2R	TCGCCAGTTATCTGACATTCTG				
uidA F	TGGTAATTACCGACGAAAACGGC	uidA	162	52	Godambe et al. (2017)
uidA R	ACGCGTGGTTACAGTCTTGCG				

Unidirectional sequence reads were performed by standard procedures and the contigs were assembled using bioedit (version 7.2.5.0) sequence program (Hall et al., 1999). The evolutionary history was inferred using the neighbour-joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the jukes-cantor method and are in the unit of the number of base substitutions per site (Jukes and Cantor, 1969). All positions containing gaps and missing data were eliminatory. Evolutionary analyses were conducted in molecular evolutionary genetics analysis 6.0 (MEGA6) (Tamura et al., 2013).

Susceptibility testing

Kirby-Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI, 2017) was applied. Briefly, a single colony of pure isolate was inoculated into a test tube containing 1 mL of nutrient broth (Oxoid, UK) and incubated overnight at 37°C. The overnight broth was then standardized to match 0.5 McFarland standard. A sterile swab stick was dipped in the standardized suspension and streaked over the surface of prepared Mueller Hilton agar plates (Oxoid LTD, Basingstoke, Hamshire, England). The antibiotic disc (Abtek Biologicals Limited Gram-negative discs); gentamicin (10 µg), ceftazidime (30 µg), cefturoxime (30 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), amoxicillin/clavulanate (30 µg), ofloxacin (5 µg) and cefixime (5 µg) were placed on the agar surface maintaining a distance of 30 mm edge to edge. The plates were incubated at 37°C for 24 h. The clear zone of inhibition was measured with a ruler to the nearest diameter and results were interpreted in accordance with Clinical and Laboratory Standards Institute guidelines (2017).

Detection of integrons by PCR

The presence of integrons in Enterobacteriaceae isolates was determined by PCR using consensus primers (Hep35 and Hep36) as described elsewhere (Su et al., 2006). Amplification was carried out using miniPCR (USA) with the following thermal cycling profile: initial denaturation at 94°C for 3 min, denaturation at 94°C for 30 s, annealing at 37°C for 30s and extension at 68°C for 30 s and a final extension at 68°C for 5 min for 30 cycles (Table 1). Amplicons were analyzed by blueGel agarose electrophoresis system (USA). Integrons were classified using restriction fragment length polymorphism (PCR-RFLP) polymerase chain reaction supplemented with gene-specific primers, while Class 1 integron was confirmed by Hep58 and Hep59 primers as described elsewhere (Rezaee et al., 2011).

RESULTS

Species identified and status of shiga toxins in *E. coli*

Three hundred and fifty-nine clinical samples were obtained, of which forty-nine Enterobacteriaceae comprising *E. coli* (45), *Proteus mirabilis* (2), *Shimwellia blattae* (1), and *Klebsiella pneumoniae* (1) were isolated from 36 (73.50%) female and 13 (26.50%) male subjects (Figure 1 and Table 2). Majority of the isolates were *E. coli* as confirmed by specific primer (Figure 2). However, *E. coli* O157:H7 strains were not detected in this study. Of the 49 isolates, 24 (48.98%), 17 (34.69%), 6 (12.24%) and 2(4.08%) were recovered from urine, stool, endocervical swab and high vaginal swab respectively (Table 2). All sequenced data were deposited in GenBank under the accession numbers MT271687-MT271693 and their phylogenetic relationship to those in GenBank was constructed (Figure 3).

Susceptibility pattern and integrons status of species encountered

All the twenty-four isolates (100.00%) obtained from urine were resistant to ceftazidime, cefuroxime, cefixime, amoxicillin/clavulanate, while 22(91.67%), 22(91.67%), 21(87.50%) and 16(66.67%) were found to be resistant to ofloxacin, ciprofloxacin, gentamicin, and nitrofurantoin respectively in the same urine sample. Also, isolates obtained from stool samples 17 (100.00%) were resistant to amoxicillin/clavulanate, cefixime, ceftazidime and cefuroxime while 16 (94.10%), 15 (88.23%), 14 (82.30%) and 7 (41.08%) were resistant to ciprofloxacin, ofloxacin gentamicin, and nitrofurantoin in that order. Likewise, isolates obtained from endocervical swab and high vaginal swab samples were equally resistant to most of

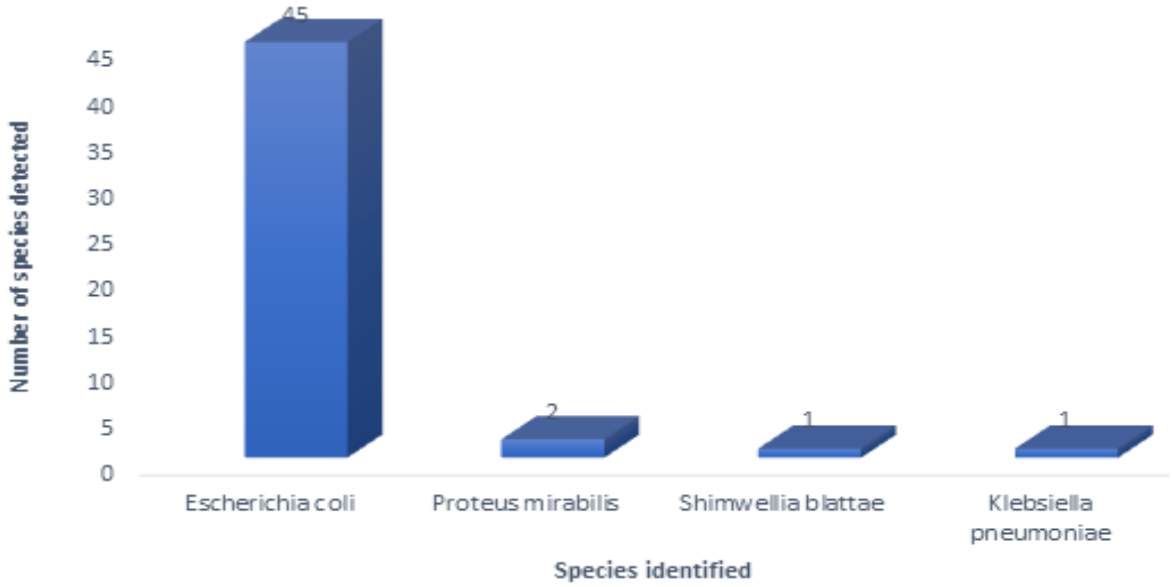

Figure 1. Distribution of species of Enterobacteriaceae obtained from clinical samples.

Table 2. Source distribution and target genes per source in studied isolates.

Sources	Number of clinical samples	Number of organisms isolated		*Integrase gene*		Class 1 integron		*E. coli* 0157:H7	
		No.	%	No.	%	No.	%	No.	%
Urine	80	24	48.98	7	29.17	7	29.17	x	0
Stool	70	17	34.69	6	35.29	6	35.29	x	0
Endocervical swab	80	6	12.24	5	83.33	5	83.33	x	0
High vaginal swab	79	2	4.08	2	100.00	2	100.00	x	0
Total	**309**	**49**	**100**	**20**					

X: Absence of target gene, no: number of target.

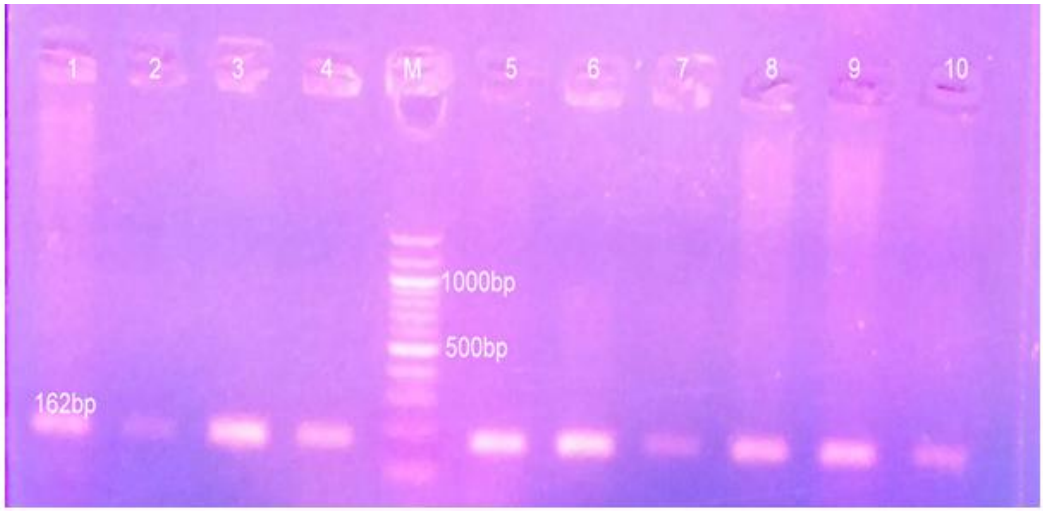

Figure 2. Electrophoregram of *E. coli* detection using uid primers. M: Molecular weight ladder (100 bp), known *E. coli* (control): 1, Isolates: 2-10.

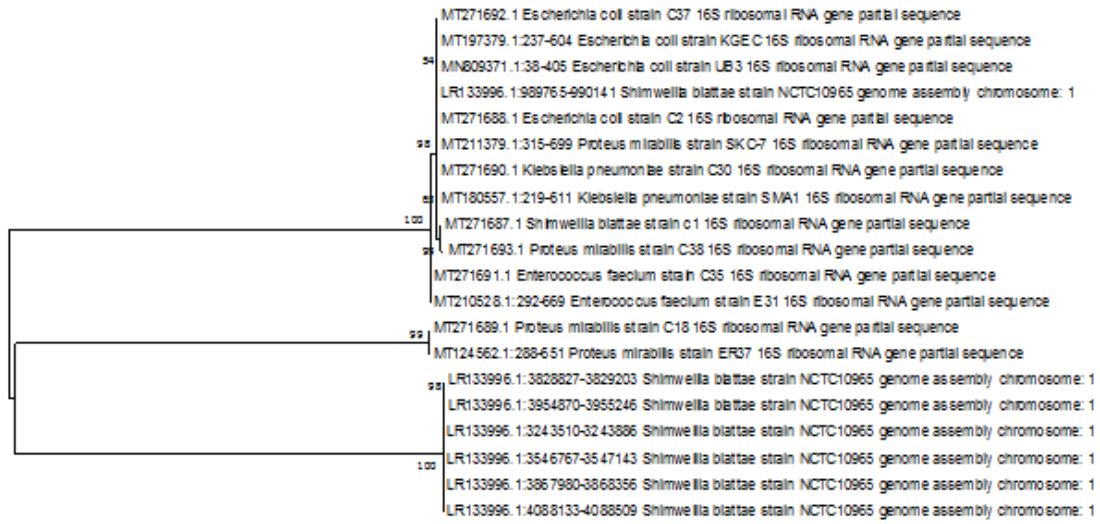

Figure 3. Phylogenetic tree illustrating the relationship between the isolates identified and their close relatives in NCBI. The evolutionary history was inferred using the Neighbour-Joining method and distances were computed using the Jukes-Cantor method. All the isolates were grouped into two clusters. Cluster: 1 E. coli C37, C2, K. pneumoniae C30, P. mirabilis C38. Cluster 2: P. mirabilis C18. Two species Enterococcus faecium were used to root tree.

the antibiotics (Figure 4). Multidrug resistance pattern showed that 29 (59.18%) of the isolates were resistant to all the antibiotics tested while 13 (26.53%), 5 (10.20%), 1 (2.04%) and 1 (2.04%) were resistant to seven, six, five and four antibiotics respectively (Figure 5). Class 1 integrons were found in 20 (40.82%) of the isolates. However, no class 2 and 3 integrons were detected in the isolates (Figure 6).

DISCUSSION

In this study, *E. coli*, *Shimwellia, Klebsiella*, *Enterobacter* and *Proteus* species were recovered from clinical samples. The family Enterobacteriaceae are usually found in the environment as well as the normal microbiota of the intestine in humans and other animals. The recovery of these species from urine, stool, endocervical swab and high vaginal swab is not surprising because members of this species remain harmlessly confined in some parts of the body. However, in weakened or immunosuppressed host, non-pathogenic strains can trigger infections that may be responsible for many illnesses in individuals and livestocks (Muhammad et al., 2011). The prevalence of Enterobacteriaceae in this work is comparable to a report by Malek et al. (2015). It is imperative to note that Enterobacteriaceae were recovered more in female than in male counterpart. This result was comparable to results obtained previously by other authors (Onyedibe et al., 2018; Ibrahim et al., 2018) in North Central Nigeria, and Saudi Arabia. The reason for high prevalence in the case of females may be attributable to the nature of their genitals which predispose them to faecal contamination when compared to their male counterpart whose relatively closed genitals prevent the establishment of pathogens.

Members of the family Enterobacteriaceae are frequently identified as etiological agents of nosocomial infections (Obeng-Nkrumah et al., 2013; Bouguenoun et al., 2016) and can cause various diseases, ranging from urinary tract infections (UTIs), pneumonia, wound infections, bloodstream infections, intestinal infections such as enteritis and diarrhea to central nervous system infections (Osman et al., 2018; Dougnon et al., 2020; Breijyeh et al., 2020).

In this study, *E. coli* was the most commonly isolated organism. This is consistent with results obtained by several authors (Tajbakhsh et al., 2015; Osman et al., 2018), but contrary to some other authors (Obeng-Nkrumah et al., 2013; Bouguenoun et al., 2016; Akbari et al., 2018). The variation may be attributable to the sample size used or species diversity in different study locations. Most of the Enterobacteriaceae detected in urine may be the primary cause of urinary tract infections. These bacteria adhere to vaginal epithelium cells, and also invade vaginal cells leading to infection (Brannon et al., 2020). Therefore, this might have accounted for their prevalence in the urine samples. Urinary tract infections are one of the most common bacterial infections caused by members of Enterobacteriaceae that affect humans in community and hospital settings which accounted for up to 88.0% cases (Park et al., 2017).

Colonization of *E. coli* in the vagina and cervix has been reported to cause a lot of diseases and illnesses to humans (Olowe et al., 2012; Kumari et al., 2016; Orish et al., 2016). In a study (Kumari et al., 2016), it was

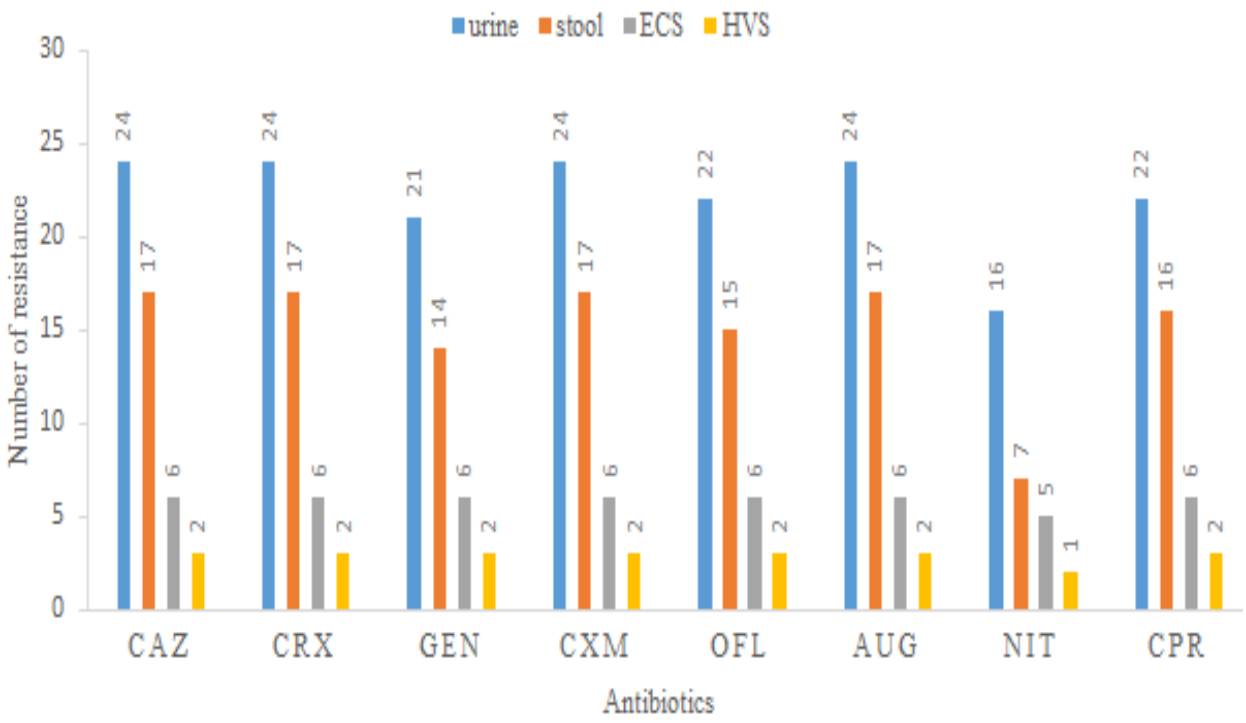

Figure 4. Resistance pattern of isolates against selected antibiotic classes. CAZ- Ceftazidime, CRX- Cefuroxime, GEN- Gentamicin, CXM- Cefixime, OFL- Ofloxacin, AUG- Amoxicillin/clavulanate, NIT- Nitrofurantoin, CPR-Ciprofloxacin

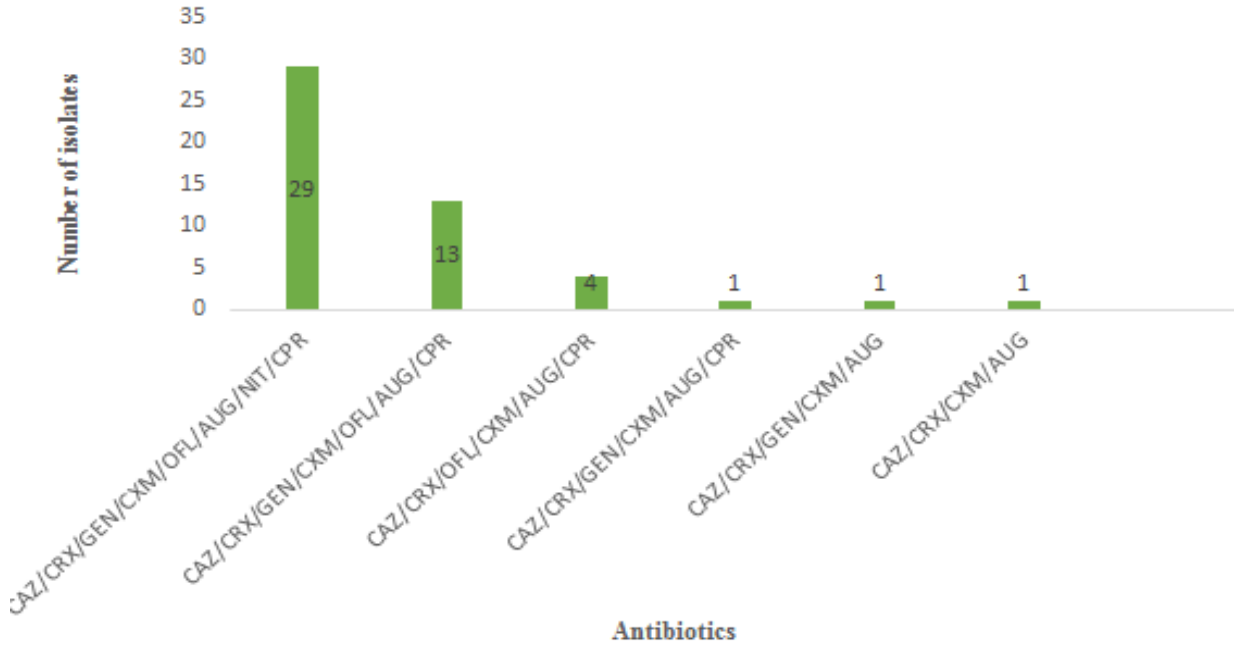

Figure 5. Multi-drug resistance profile of isolates obtained from clinical samples. CAZ- Ceftazidime, CRX- Cefuroxime, GEN- Gentamicin, CXM- Cefixime, OFL- Ofloxacin, AUG- Amoxicillin/clavulanate, NIT- Nitrofurantoin, CPR-Ciprofloxacin.

observed that the most predominant Gram-negative organisms responsible for pelvic inflammatory disease and infertility in women were *E. coli* and *Klebsiella*. In the same trend, studies amongst patients with suspected

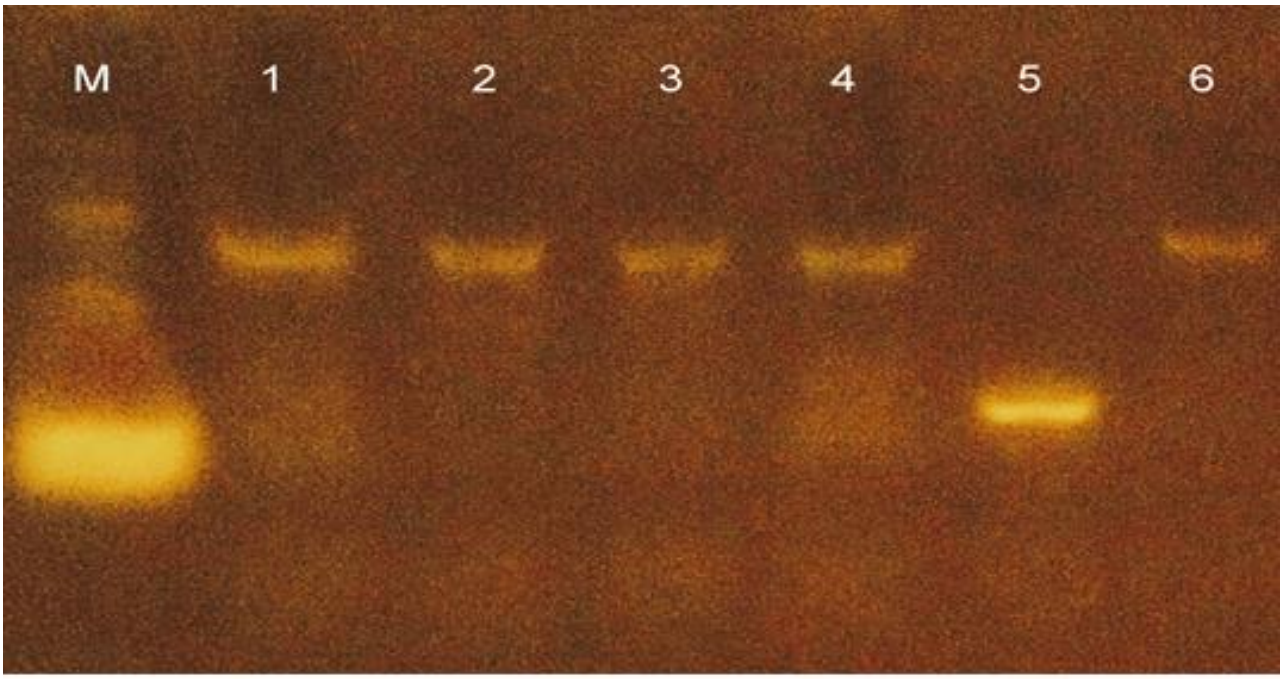

Figure 6. Electrophoregram of class 1 integron. M: Molecular Weight Marker (100bp), Isolates: 1-6.

pelvic inflammatory disease in Osogbo, Nigeria, revealed that 70% of female genitals were colonized by *E*. *coli* (Olowe et al., 2012). Thus we can say that the prevalence of *E*. *coli* over other species in the urine, high vaginal swab and endocervical swab could be an indication that the patients might have been suffering from either of the aforementioned infections.

High level resistance of isolates was observed against ceftazidime, cefuroxime, cefixime and amoxicillin/ clavulanate, ofloxacin, ciprofloxacin and gentamicin. This is in harmony as reported by Iliyasu et al. (2018). Most of the antibiotics we used have no bactericidal effect on the strains encountered. This observation is consistent with previous studies (Omololu-Aso et al., 2017; Ibrahim et al., 2019), but is in contrary to the report of other authors (Ogidi and Oyetayo, 2013; Waseem et al., 2015).

The high level of resistance may be attributed to antibiotic pressure in clinical settings. Kibret and Abera (2011) reported high level resistance of *E*. *coli* to amoxicillin (86.0%), but highly susceptible to nitrofurantoin (96.4%), norfloxacin (90.6%), ciprofloxacin (79.6%), erythromycin (89.4%) and (72.6%) tetracycline. The variation in resistance could be attributed to the different strains of bacteria encountered as well as different antibiotic pressures in the studied environments. In addition, antibiotic abuse associated with self-medication which often results in inadequate dosage could have contributed significantly to this resistance profile (Ezeamagu et al., 2018). Many factors affecting microbial resistance phenotype have been highlighted elsewhere (Corona and Martinez, 2013). The resistance of the isolates against nitrofurantoin was on the high side and is similar to Jafri et al. (2014) where (52.5%) of the organisms were resistant to the same antibiotics (Jafri et al., 2014). Nitrofurantoin is one of the most appropriate antibacterial agents for empirical therapy of UTIs because it is highly concentrated in the urine and it is administered orally. However, high level of resistance observed is a signal that in the nearest future treatment failure due to Enterobacteriaceae infections will be anticipated. Therefore, increasingly presence of these antibiotics in the clinical settings will result in rapid development of resistance (Munita and Arias, 2016; Tuem et al., 2018; Aslam et al., 2018).

The sale of medicines without a prescription is an important regulatory issue in the abuse of antibiotics. It has been reported that bacteria acquire resistance by horizontal gene transfer of mobile genetic elements and that high usage of the antibiotics influences the selection of existing resistance mechanisms (Stokes and Gillings, 2011). Multidrug resistance has serious implications for the empiric therapy of infections caused by bacteria such as *E*. *coli*, *Klebsiella*, *Enterobacter* and *Proteus* species especially those that harbour integrons.

Integrons play an important role in antibiotic resistance, and they are able to capture, integrate, and express those gene cassettes encoding antibiotic resistance (Park et al., 2018; Partidge et al., 2018). We found integrons belonging to class 1 in 40.81% of the isolates encountered while class 2 and 3 integrons were absent. Also, the prevalence rate of integrons is comparable to several studies (Chang et al., 2000; Essen-Zandbergen

et al., 2007; Japoni et al., 2008; Muhammad et al., 2011; Kor et al., 2013; Tuem et al., 2018; Ibrahim et al., 2019), but differed from results elsewhere (Daikos et al., 2007; Fuentes et al., 2013; Hadizadeh et al., 2017).

The variation could be attributed to geographical location and environment. Few studies in Nigeria have reported the presence of integrons in clinical and environmental isolates. Odetoyin et al. (2018) detected class 1 (31%) and class 2 (4%) integrons in faecal *E. coli* strains of mother-child pairs in Osun State, Nigeria. Class 1 integrons (57.4%) were also detected in *P. aeruginosa* isolated from clinical isolates in South-West Nigeria (Odumosu et al., 2013).

Adesoji et al. (2017) identified 27.3% class 1 integrons in multidrug-resistant *Pseudomonas* from water distribution systems in South-western, Nigeria. It is likely that integrons Class 1 are frequently detected among clinical isolates than environmental isolates in Nigeria. The presence of integrons has no association with the degree of resistance as observed in this work. Other authors (Dakic et al., 2007; Japoni et al., 2008) had a slight association in the degree of resistance although majority are not statistically significant in terms of resistance pattern.

Conclusion

It can be inferred from this work that a large proportion of the Enterobacteriaceae encountered were multi-drug resistant and possessed integrons. Consequently, there is a need for proactive antibiotic surveillance system in both healthcare and community settings with a view to reducing the incidence and spread of antibiotic resistance genes between different species of bacteria.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank all personnel at the University of Abuja Teaching Hospital, Gwagwalada for their technical assistance during the execution of this project.

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Vol. 13(1), pp. 11-19, January-June 2021
DOI: 10.5897/JMA2020.0440
Article Number: 15B8C1E66572
ISSN: 2141-2308

http://www.academicjournals.org/JMA

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Journal of Microbiology and Antimicrobials

Full Length Research Paper

Pathogenicity, epidemiology and antibiotic resistance of *Vibrio cholera* strains in some West African Countries: A Systematic Review

Eliane Akpo, Tamegnon Victorien Dougnon*, Alidehou Jerrold Agbankpe and Honore Sourou Bankole

Research Unit in Applied Microbiology and Pharmacology of Natural Substances, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 P. O. Box 2009, Cotonou, Benin.

Received 10 December, 2020; Accepted 30 March, 2021

Cholera is an epidemic disease and a real public health problem throughout the world, particularly in West Africa. This study provides a comprehensive overview of the pathogenicity, epidemiology and *Vibrio cholerae* strains's antibiotics resistance in West Africa. A literature review was conducted online in English using the keywords "Cholera", "*Vibrio cholerae*" "West Africa", "Epidemiology", "Antibiotic resistance". These keywords were entered into using electronic databases such as PubMed, Google Scholar, Scopus and Elsevier and articles were used according to the reliability of their sources, study areas, and subjects. This review was based on the collected data from different databases. One hundred and twenty-three articles were identified. After the initial and final sorting of the collected data in order to eliminate duplicate copies, eighty-three were retained while seventy articles were selected, respectively, for this review. Though some studies had recommended for a system of monitoring cholera in West African countries, nevertheless, there is the need to create more awareness. Furthermore, hygienic practices and environmental wastes management in these countries need to be improved.

Key words: Cholera, epidemiology, *Vibrio cholerae*, antibiotic resistance, West Africa.

INTRODUCTION

Since 1817, seven Cholera pandemics whose causative agent is *Vibrio cholera* have been documented (Webb, 2019). Cholera has continued to be a threat to the health of many communities worldwide. Annually, about 3 to 5 million people are affected by cholera and 100,000 to 120,000 lives are lost (WHO, 2014; Ali et al., 2012). Since the first pandemic which occurred in West Africa (Webb, 2019), some outbreaks have been frequently

*Corresponding author. E-mail:urmaphaepac@hotmail.com.

reported in Benin, Ghana, Nigeria, Ivory Coast and Togo (WHO, 2016). However, only a few small-scale studies have examined the dynamics of recent cholera outbreaks in West Africa. Overall, cholera epidemics throughout the study region have shown different characteristics depending on the country. In West African countries like Benin and Togo, cases of cholera occur every year, but with a relatively low incidence (Moore et al., 2018; Landoh et al., 2013). However, many countries in the northwest, including the Gambia, Senegal, and Mauritania, have registered multi-year cholera epidemics with high incidence (Manga et al., 2018). Several epidemics have occurred following natural disasters (flooding, earthquake, etc.) and major population displacements. This is the case of Senegal in 2004-2006 (Manga et al., 2018). Nowadays, cholera remains endemic in Asia and Africa, due to the shortages in sanitary systems of these countries coupled with the social-cultural behavior of populations, as well as the lack of hygienic practices and environmental sanitary activities (Sule et al., 2017). Recent studies carried out in some West African countries, including Nigeria, Republic of Benin, Togo, Ghana, and Ivory Coast, for a period of 20 years (1987-1994) on the relationships between climate, inter annual variability and cholera revealed temporo-spatial synchrony between cholera incidence and rainfall in all the countries besides Ivory Coast (Boeckmann et al., 2019). Thus, cholera is a waterborne disease caused by ecological factors (Ruenchit et al., 2019). During the seventh pandemic, antibiotic resistance as well as the virulence of *V. cholerae* strains increased and another variant of the cholera biotype occurred (Safa et al., 2010).

The global spread of antibiotic-resistant strains of *V. cholerae* is now threatening the effective treatment and control of cholera, particularly in low- and middle-income countries. Current evidence shows that cholera represents a serious threat to the African continent (Sambe-Ba et al., 2017). Because of this situation in West African countries and the worrying public health phenomenon of antimicrobial resistance (WHO, 2016), it has become very necessary to create more awareness on the resistive nature to antibiotics of *V.cholera* strains particularly in West African countries. The present article review was aimed at identify, through a review of related literatures, information on pathogenicity, epidemiology and antibiotics resistance of *V. cholerae* strains in West Africa.

METHODOLOGY

Methods of search and article selection

The review work was conducted using the following keywords “Cholera”, “*V. cholerae*” “West Africa”, “Epidemiology”, “Antibiotic resistance”. These keywords, with the use of PubMed, Google Scholar, Scopus and Elsevier, assisted in collating reliable articles based on source, study area, and subject. The search strategy was based on three components: (1) Epidemiology of cholera in West Africa; (2) *V. cholerae* in humans; (3) Characterization of *V. cholerae* in food, environment and feces; (4) Antibiotic resistance of isolated *Vibrio cholerae* strains. The following descriptors and Boolean operators were used, while searching for articles, no language or timeline restrictions were applied. The initial selection was based on the title and summary of all articles found. Duplicate articles were eliminated, and all potentially relevant articles were uploaded for eligibility assessment.

Data extraction, exclusion, and inclusion criteria

The exclusion of the articles was based on well-defined criteria, as follows: (1) studies on Vibrio strains, and (2) studies that were non-journal papers such as editorials, dissertations and thesis, book, editors’ letters, Master or Doctoral theses, book chapters, and articles whose complete text was unavailable. Reference lists of the selected articles were also examined to find potentially relevant documents. The criteria used for inclusion were based on articles relevance on: epidemiology of cholera; cholera and transmission of *Vibrio cholera* through food, as well as food products and the environment, and antibiotic resistance of strains of *Vibrio cholera*. Such criteria were defined to fulfill the proposed objective: the epidemiology of cholera in West Africa, the transmission of *V. cholerae* through food, the environment and the antibiotic resistance of these strains. Qualitative data were extracted from all the selected articles. The data extraction was classified as follows: (1) characteristics of the publication: author, year, journal, and country; (2) characteristics of the *V. cholerae*: source; antibiotic resistance and main results of the study.

RESULTS

This systematic review is the result of data collection carried out in different databases. One hundred and thirty-three articles were identified. After a first sort duplicate articles were eliminated, and all potentially relevant articles were uploaded for eligibility assessment. We, therefore, retained 83 articles. An in-depth reading of the articles led to the second selection of 70 articles for this study. Figure 1 shows the item selection diagram according to the PRISMA statement.

Epidemiology of Cholera in West Africa

In Africa, the studies of Ramamurthy et al. (2019), clearly describe the epidemiology of cholera in Africa. Seven cholera pandemics have been experienced globally and it continues to cause outbreaks locally and regionally across the African continent (WHO, 2019; Moore et al., 2018). According to WHO (2019), there were about 1.3 to 4.0 million reported cases and about 21,000 to 143,000 recorded deaths as a result of cholera globally in 2018. Between 1970 and 2011, Democratic Republic of Congo,

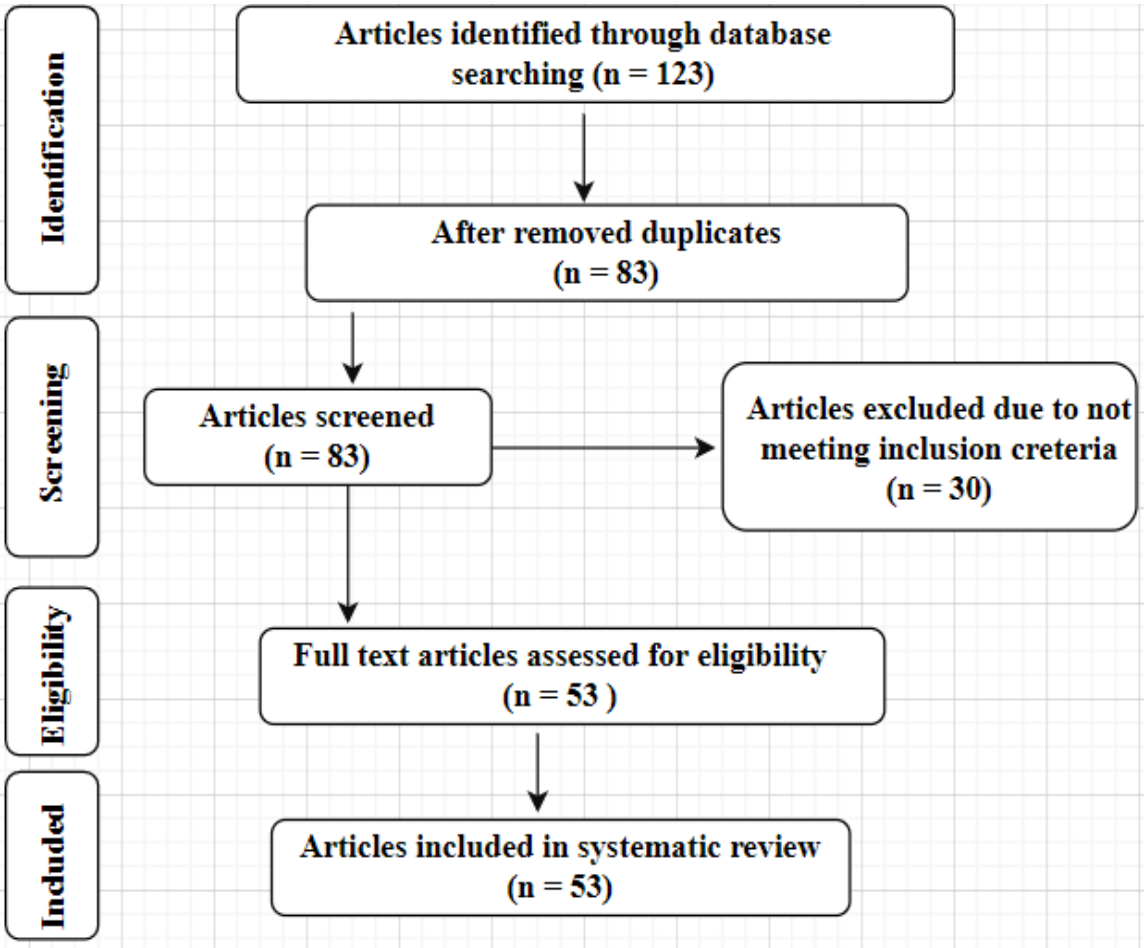

Figure 1. PRISMA model study design process. n: number of articles.

Mozambique, Nigeria and Tanzania recorded pooled estimate of about 260,000 to 390,000 cases of cholera diseases and 11,000 to 25,000 deaths (Mengel et al., 2014). The genomic approach has made it possible to identify cases of cholera transmission from South Asia to Africa (T1, T3-T13) and from Africa to America (T2) or Asia (T13). Of these, T1-T5, T6-T8, T9-12 are wave 1, wave 2, and wave 3 respectively (Weill et al., 2017). T1 occurred in the 1970s in southeastern Asia and follow-up in the Middle East and Russia. In Europe, only imported cases of cholera have been reported in the recent past. European T1 isolates from the early 1970s originated in West and North Africa. T2 (1989-1991) was responsible for the spread of cholera from West Africa. The South Asian origin T7 (1982-1984) was detected in isolates from several epidemics in North and West Africa. The T8 subline from the Middle East was associated with epidemics in South Africa in 2001-2002. Outbreaks in Zimbabwe in 2008-2009 were associated with T8 and T11. Most of the African countries were effected with T9–T12 from 1990–2014 and these sub-lineages originated in South Asia. In West Africa, number of reported cases increased to 16,088 compared to 3,074 in 2010 (Goita, 2014). Other isolates from western and southern Europe in the early 1970s were found to originate from West or North Africa. After being introduced into West Africa in 1970, cholera was detected several times in that region, with extensions into the Gulf of Guinea region and the Lake Chad basin :T7 (dates of introduction: 1982 to 1984), T9 (1988 to 1991), and T12 (2007) (Weill et al., 2017).

Ghana has experienced numerous epidemics since the first outbreak reported in 1970 (WHO, 2019; Noora et al., 2017). Over the past two decades, the country has reported an average of 3,066 (range: 50-10,628) cholera cases with a fatality rate of 1.7%, although WHO (2013) estimated that over 40,000 cases occurred every year during the outbreaks (Noora et al., 2017). The epidemic

in 2012, which recorded 9,548 cases in 9 regions and killed 100 (WHO, 2013, Ghana Health Service, 2013). With more than 28,975 cases and 243 deaths, the cholera outbreak from 2014 to 2015 in Ghana was the worst outbreak on record in the country. Later in 2016, a small wave of the disease was recorded in the central region of Ghana, accounting for 596 cases (UNICEF-Ghana, 2016).

Cholera disease is said to be endemic, epidemic and it is frequently occurring in Nigeria (Cholera annual Report, 2011). According to surveillance data obtained from the Epidemiology Division of the Federal Ministry of Health, between January 2004 and December 2008, cholera outbreaks were reported in 12 states in Nigeria with 74,881 cases and 1,387 deaths (Gidado et al., 2018). The 2010 cholera epidemic was the largest outbreak in Nigeria since 1991, when 59,478 cases and 7,654 deaths were reported (WHO, 2010; Dalhat et al., 2014). From September to December 2013 in Nigeria, a total of 6,600 cholera cases, including 229 deaths, were reported in 94 local authorities (LGAs) in 20 states (including Kaduna State). Kaduna State has been in the forefront during the latest cholera outbreaks in Nigeria. On August 1, 2015, an epidemic of suspected cholera cases was reported in Zaria LGA. With over 40 cases and over 10 registered deaths (WHO-Cholera, 2016; WHO, 2016b).

After the seventh cholera pandemic in 1970 caused by V.cholerae O1 biotype El Tor, Guinea-Bissau, located along the West African coast in northern Guinea, reported outbreaks for the first time in 1987 (Dalsgaard et al., 2000; WHO, 1987), which was followed by an epidemic in 1994 which spread in early 1995 (Dalsgaard et al., 2000). The epidemics of 1987 and 1994–1995 were determined through phenotypic and genotypic analyzes to be caused by different O1 strains, with the 1994-1995 epidemic strain probably having been introduced by fishermen or travelers from Guinea (Dalsgaard et al., 2000). The most recent outbreak in Guinea-Bissau began in October 1996, spread throughout 1997, and included a total of 26,967 reported cases, with an increased case fatality rate (Dalsgaard et al., 2000).

Togo has experienced endemic cholera for at least the past 40 years, mainly in the coastal region (Constantin et al., 2007; Bockemühl and Meinicke, 1976; Bockemühl and Schröter, 1975; Amedome et al., 1971). Outbreaks have sometimes been large, with case fatality rates reaching 10% (Bockemühl and Schröter, 1975). More recently, cholera continues to be rampant in Togo, including in 2011, when Togo experienced hundreds of cases and over 30 deaths (Landoh et al., 2013). In addition to the risk of cholera linked to diseases located in Togo itself, it is possible that Togo is exposed to the risk of cholera imported from neighboring countries, as the disease is endemic in a large part of West Africa (Landoh et al., 2013; Gbary et al., 2011; Thompson et al., 2011; UNICEF, 2011). The current evaluation of surveillance data from the National Ministry of Health was undertaken as part of Togo's participation in the African Cholera Surveillance Network (Africhol; available at: http://www.africhol.org; consulted on 4 June 2020) to describe the epidemiology of cholera, including the suspected incidence, to better inform public health decision-making.

In Benin, the lakeside commune of Sô-Ava, which is directly connected to Nigeria via Lake Nokoue and Yewa River, reported cholera outbreaks every year since 2010 and was often the first and hardest-hit commune. In 2013, Sô-Ava reported 40% of cholera cases in 2013 and 30.4% of cases in 2014 (Manga et al., 2018). Cotonou, the economic capital of Benin, was affected by cholera epidemics in 2010, 2011 and 2013, in neighborhoods characterized by fishing activity and significant population movements (WHO, 2019).

In many countries of northwestern West Africa, such as The Gambia, Senegal and Mauritania, have experienced marked lulls for several years (Somayyeh et al., 2018; Aidara et al., 1998; Roquet et al., 1998). Many major epidemics have erupted in the wake of violent civil conflicts that have generated a humanitarian and public health crisis or massive population movements like that of Senegal in 2004-2006 (WHO, 2019; Somayyeh et al., 2018, Ekra et al., 2009) (Table 1). The vast majority of cases have also been reported in large cities following increased rainfall (Somayyeh et al., 2018, Ekra et al., 2009; Rebaudet et al., 2014; Wendo, 2003). Although many studies have been limited to a single epidemic / neighborhood or to a short period, common risk factors have been found across the region: overcrowded living conditions, poor sanitation, and limited access to clean water (Palma et al., 2011; Gbary et al., 2011).

Transmission of *V. cholerae* in Africa

According to Weill et al. (2017) recurrent transmissions have been observed for cholera epidemics in Africa. Different sub-pandemic ages of the seventh *V. cholerae* El Tor (7PET) cholera pandemic from Asia were repeatedly introduced into two main regions: West Africa and East/Southern Africa. Epidemic waves then propagated regionally, in some instances spreading to Central Africa, over periods of a few years to 28 years. Only two notable instances of subline-age exchange between these two circulation hotspots were identified: The spread of a subline-age between Angola and Mozambique during the Portuguese colonial war in 1970s (Echenberg, 2011) and the spread of a subline-age from the African Great Lakes Region to the western part of the Democratic Republic of Congo (Rebaudet et al., 2013)

Table 1. The number of suspected cases of cholera reported in each country included in the study per year.

Country	Suspected cholera cases reported							
	2009	2010	2011	2012	2013	2014	2015	Total
Coastal West African countries included in the epidemiological study								
Ivory Coast	5	32	1261	424	56	235	199	2212
Guinea	42	0	3	7350	319	1	0	7715
Benin	74	983	775	668	528	832	0	3860
Togo	218	72	33	61	194	262	35	875
Liberia	1070	1546	1146	219	92	44	0	4117
Sierra Leone	0	0	0	23124	377	0	0	23501
Ghana	1294	438	10387	9563	20	28944	692	51338
Guinea-Bissau	5	0	0	3068	969	11	0	4053
Senegal	4	3	5	1	0	0	0	13
Mauritania	0	0	46	0	0	0	0	46
The Gambia	0	0	0	0	0	0	0	0
Countries neighboring the study region								
Burkina Faso	0	0	20	143	0	0	0	163
Nigeria	13691	44456	23377	597	6600	35996	5290	130007
Niger	0	1154	2324	5284	585	2059	51	11457
Mali	0	0	2220	219	23	0	0	2462

Source: WHO (2016a).

and the Central African Republic via the Congo River and its tributaries in 2011 to 2012.

V. cholerae resistance mechanisms

The resistance among *V. cholerae* strains is attributed to target modifications or acquisition of resistance genes from mobile genetic elements. The major source of antibiotic resistance in cholera pathogens among the various mobile genetic elements are the Integrative conjugative elements (Banerjee et al., 2014). Bio mechanical mechanisms that the micro-organisms use to express resistance against antimicrobial agents include; drug alteration or inactivation by production of enzymes such as β- lactamases that act by hydrolyzing the β lactam ring, aminoglycoside-modifying enzymes and chloramphenicol acetyltransferases; modification of drug binding sites, changes in cell permeability thus leading to a reduction in intracellular drug accumulation, for instance presence of efflux pumps which expel the drugs and also loss of porin, a protein present on the outer membrane of gram negative bacteria; biofilm formation (Munita and Arias, 2016).

Antibiotic resistance of *V. cholerae* in West Africa

According to Marin et al. (2013) Cholera outbreaks in Nigeria are associated with multidrug resistant atypical El Tor and Non-O1/NonO139 *V. cholera* (Marin et al., 2013). We can understand that antibiotic multidrug resistance is becoming increasingly common among the atypical *V. cholerae* strains, mostly associated with acquisition of genes and/or modification in the antibiotic target genes. They remark that the current O1 Nigeria strains were resistant to streptomycin, trimethoprim and sulfonamides (Table 2). In *V. cholerae*, these resistances are frequently associated with class 1 and 2 integrons and SXT element, which is a *V. cholerae*-derived integrating and conjugative element (ICE). Thus, we investigated the presence of these elements in the Nigeria strains. All the current O1 strains harbor an ICE element, determined by the presence of the SXT integrase gene. No evidence was found for the presence of class 1 and 2 integrons (Opajobi et al., 2004; Okeke et al., 2001). All these genes were identified, explaining the resistance profile of the current O1 strains. Majority of the 2009/2010 Nigeria O1 strains showed reduced susceptibility to ciprofloxacin as well as resistance to nalidixic acid (Table 2).

Dalsgaard et al. (2000) from Guinea Bissau demonstrated resistance to ampicillin, aminoglycosides, cotrimoxazole and tetracycline. Only colistin remained effective from their study. They also demonstrated that resistant isolates possessed a multiresistance transmissible plasmid that encoded trimethoprim (dhfrXII) and aminoglycoside resistance.

Table 2. Antibiotic resistance of *V. cholerae* O1 strains responsible for cholera in West Africa.

Countries	Years of outbreak	Number of *V. cholerae* strains	Antibiotic resistance of strains of *Vibrio cholerae*	Authors and year of publication
Ghana	2012-2015	168 strains of *Vibrio cholerae* isolated from feces of hospitalized patients: 154 serogroup O1 and 14 non O1/O139 serogroup. 151 serotype Ogawa and 3 Inaba	Sulfamethoxazole-Trimethoprim, Nalidixic acid, Azithromycin, Gentamicin and Flucloxacillin.	Danso et al. (2020)
Ghana	2015-2016	51 strains of *Vibrio cholera* O1. 40 strains were isolated from cholera patients between 2014-2015 and 11 strains were isolated from environmental.	92.5% of clinical isolates and 18.2% of environmental isolates were resistant to Erythromycin. 72.5% of clinical isolates and 27.3% of environmental isolates were resistant to Nalidixic acid.	Abana et al. (2019)
Nigeria	2009-2010	15 strains of *Vibrio cholerae* O1 and 5 non O1/O139.	Streptomycin, Trimethoprim-Sulfamethoxazole, Sulfonamides, Nalidixic acid, Chloramphenicol	Opajobi et al. (2004)
Nigeria	2007-2013	115 strains of *Vibrio cholerae* O1. 103 strains among serogroup Ogawa and 12 Inaba; 92 strains isolated from clinical samples and 23 strains from environmental samples.	8 to 100% of these strains were resistant to Nalidixic acid, 4 to 100% of strains to Streptomycin and 4 to 100 % of strains to Trimethoprim-Sulfamethoxazole	Adewale et al. (2016)
Guinea-Bissau	1987; 1994-1995	19 strains of *Vibrio cholerae* O1. 5 strains were isolated in 1987 and 14 strains isolated in 1994-1995.	Only strains isolated in 1987 were resistant to Polymycin B. The strains isolated in 1994-1995 were resistant to Polymycin and Trimethoprim-Sulfamethoxazole	Dalsgaard et al. (1996)
Togo	2008 to 2011	58 strains of *Vibrio cholerae* O1: 12 strains in 2008, 11 strains in 2009, 24 strains in 2010 and 11 strains in 2011.	All strains isolated in 2008 were resistant to Erythromycin, Chloramphenicol and Trimethoprim-Sulfamethoxazole. Regarding strains isolated in 2009, they were resistant to Tetracycline, Erythromycin, Chloramphenicol and Trimethoprim-Sulfamethoxazole. About strains isolates in 2010, they were resistant to Ampicillin and Trimethoprim-Sulfamethoxazole. For 2011 isolates, they were resistant to Ampicillin, Tetracycline, Chloramphenicol, Nalidixic Acid and Trimethoprim-Sulfamethoxazole.	Landoh et al. (2013)

Okeke et al. (2001) investigated an outbreak of acute gastroenteritis from Niger state, north-central Nigeria, where eight *V. cholerae* organisms were isolated. They all had the O1-serogroup and El Tor biotype. All of them were sensitive to tetracycline but resistant to trimethoprim, sulphonamide, spectinomycin and chloramphenicol, detected 34 strains of *V. cholerae* in Jos University Teaching Hospital (Nigeria) over a one-year period (WHO, 1987). They were all of the O1 serogroup, El Tor biotype and Inaba serotype. They were all resistant to chloramphenicol, ampicillin, cloxacillin and penicillin G, but sensitive to tetracycline, ofloxacin and erythromycin. A study done by Quilici et al. (2010) using the *V. cholerae* isolates from the September/October 2009 outbreak of acute watery diarrhea in north-eastern Nigeria implicated the serogroup O1 of the El Tor biotype and Ogawa serotype as the causative serotypes (Quilici et al., 2010). The toxigenic genes of ctxA and ctxB were elaborated, in addition to detected mutations in the genes responsible for quinolone resistance. All of them were resistant to trimethoprim-sulphamethoxazole, ciprofloxacin, sulphonamide and nalidixic acid. All the isolates were resistant to tetracycline, but moderately susceptible to chloramphenicol and ampicillin (Quilici et al., 2010).

In Senegal, the study of Sambe-Ba et al. (2017) identified atypical El Tor *Vibrio cholerae* O1 Ogawa that were resistant to streptomycin and cotrimoxazole. An increasing trend of resistance to cotrimoxazole was observed from many studies (Sambe-Ba et al., 2017). This is worrisome, because, until now, cotrimoxazole was considered the drug of choice against *V. cholera* (Table 2).

DISCUSSION

In addition to the seven cholera pandemics

recorded around the world and Africa in particular, local and regional outbreaks continue to be recorded on the African continent (WHO, 2019; Moore et al., 2018). The genomic approach has made it possible to identify the typical strains responsible for pandemics in Africa. Among these typical strains, strain T7 was reported between 1982 and 1984; strain T9 (1988 to 1991); strain T12 in 2007 and strains T8 and T11 (2008 to 2009) reported in Zimbabwe (Weill et al., 2017; Goita, 2014). This indicates that there were the same strains which had ravaged the entire continent, and responsible for the different epidemics. This is justified by the fact that the epidemic episodes take place in practically the same periods in each of the countries. In West Africa many countries face frequent epidemics of cholera. Ghana experienced the worst cholera outbreak from 2014 to 2015 with more than 28,975 cases and 243 deaths (UNICEF-Ghana, 2016). In 2010, Nigeria experienced its largest cholera epidemic with 59,478 cases and 7,654 deaths (WHO, 2010; Dalhat et al., 2014). Guinea, Togo, Benin, Gambia, Senegal and Mauritania have also experienced cholera epidemics since 1970 to date (Dalsgraard et al., 2000; Landoh et al., 2013; Manga et al., 2018; Somayyeh et al., 2018). The countries of West Africa face the same realities due to extremely large population, lack of sanitation infrastructure, lack of potable water, the majority of the population illiterate. Risk factors for cholera epidemics such as overcrowded settlements, lack of hygienic practices and sanitation infrastructures, and limited access to potable water are common in the West African zone (Luquero et al., 2011; Gbary et al., 2011). This is justified by the fact that cholera epidemics occur in West Africa countries of almost every year.

Resistance of *V. cholera* strains to antibiotics is due to the acquisition of resistance genes from integrative conjugative elements (Banerjee et al., 2014). In West Africa, the strain of *V. cholerae* responsible for cholera epidemics is the atypical El to strain of serogroup O1. This strain which circulates in the region is multidrug resistant and its resistance profile is practically the same in West African countries (Marin et al., 2013). In Ghana, Danso et al. (2020) and Abana et al. (2019) showed that from 2012 to 2016, nearly 75% of *V. cholerae* O1 strains isolated from the feces of hospitalized patients were resistant to Sulfamethoxazole-Trimethoprim, 92.5% were resistant to Erythromycin and 72.5% with nalidixic acid. In Nigeria, from 2007 to 2013, virtually all strains of *V. cholerae* O1 isolated from clinical and environmental specimens were resistant to Sulfamethoxazole-Trimethoprim, nalidixic acid and streptomycin (Adewale et al., 2016). In Togo, Landoh et al. (2013) presented similar results with the resistance of all strains of *V. cholerae* O1 isolated from 2008 to 2011 from clinical samples to Sulfamethoxazole-Trimethoprim, Erythromycin and nalidixic acid.

Conclusion

Cholera continues to be a real public health problem that is difficult to manage for West African countries sanitary system. This is due to the correlation between its epidemiological impact and the precarious hygiene of the population. For a more efficient fight, a mixed strategy based on sanitation to reduce the risks of contamination and on the development of more effective therapies to circumvent antibiotic resistance is required.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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