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Airborne bacterial diversity and antibiotic resistance patterns in operating theatre and paediatric wards in Botswana hospitals

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There is presently no information on the occurrence, abundance and diversity of airborne bacteria in Botswana hospitals. There is also growing concern in the global spread of antibiotic resistant bacteria that continue to emerge and pose serious challenge to human health. This study was aimed at determining the occurrence, relative abundance and diversity of airborne bacterial species and their antibiotic resistance patterns. Correlation between the meteorological conditions and the bacterial concentrations was also determined. Air impaction method was applied for the collection of airborne bacteria on selective media, antibiotic resistance was determined by screening isolates for resistance phenotypes and polymerase chain reaction (PCR) was applied for detection of resistance genes to clinically relevant antibiotics. The assessment of total airborne bacteria in hospital units revealed high abundance of airborne bacteria in paediatric wards than in the operating theatres. The highest bacterial concentrations were observed at the paediatric wards in Palapye (PPH; 7.9 × 10² CFU/m³), Maun (LMH; 6.4 ×10² CFU/m³) and Francistown (NRH; 5.8 ×10² CFU/m³). Diverse airborne bacterial species were observed with high concentration recorded for Pseudomonas species in all three hospitals’ units. The frequency of antibiotic resistance genes detected in bacterial isolates were; dfr1 (36%), mph(A) (26%), ermC (12%), strB (10%) and intI1 (16%). The study provide evidence suggesting air in hospitals’ units as a hotspot for potentially pathogenic bacteria and antibiotic resistance genes, hence the need to develop surveillance tools for monitoring the movement of airborne bacteria in hospitals in order to mitigate possible spread of nosocomial infections.

Key words: Airborne bacteria, antibiotic resistance genes, operating theatre, paediatric, human health.

INTRODUCTION

Airborne microorganisms are widespread in the atmosphere, contributing to various health aspects in

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human and the ecosystem (Zhai et al., 2018). The natural sources of airborne microorganisms such as bacteria, viruses, fungi include water, soil and vegetation (plants), anthropogenic sources such as wastewater and faecal material from human and animal activities also contribute to the concentration of airborne microorganisms in the atmosphere (Zhao et al., 2014, Smets et al., 2016). Built environments (houses and community spaces) are major sources of airborne bacteria associated with adverse human health risks, outdoor bacteria frequently enter indoors of buildings through open doors and windows (Prussin et al., 2015; So et al., 2017). Microbial contamination of indoor hospital environments like operating theatre and paediatric wards may pose as a health risk to patients because of confined spaces. Indoor spaces may harbour aerosols containing microbes and allow to build to infection levels (Augustowska and Dutkiewicz, 2006). Persistent exposure to pathogenic airborne microorganisms leads to severe respiratory disorders, allergic reactions, hypersensitivity pneumonitis and sick building syndromes (Görny et al., 2002; Fracchia et al., 2006; Griffin et al., 2003; Yassin and Almouqatea, 2010). The most susceptible population to airborne nosocomial infections are children, the elderly and immunocompromised patients.

The atmospheric microbial contamination in hospital environment, mainly in operating theatre wards continue to increase prevalence of nosocomial infections which lead to high mortality rates among hospitalized patients going through post-operative surgery (Weigelt et al., 2010; Hailemariam et al., 2016; Spagnolo et al., 2013). Findings from these studies indicated that pathogenic microorganisms in operating theatres are acquired from exogenous sources such as the operating theatre surrounding environment, surgical personnel and equipment brought to the sterile field during operation. In previous studies, the predominant bacterial species isolated from operating theatre wards were Staphylococcus species (the leading cause of surgical site infections, SSI), Enterococcus, Escherichia coli and Pseudomonas aeruginosa (Wolcott et al., 2009; Hailemariam et al., 2016). In addition to the operating theatre, the paediatric wards have also been widely studied for their role as source of bacterial infections. The most prevalent bacterial genera isolated in paediatric wards was also Staphylococcus aureus (Okten and Asan, 2012; Qudiesat et al., 2009). Another similar study conducted in a paediatric ward was by Coetzee et al. (2013), which indicated the highest isolation of P. aeruginosa. Serratia marcescens, Acinetobacter and Klebsiella species in paediatric wards (Behzadnia et al., 2014).

Antibiotic resistance has caused concern in hospitals where bacterial infections are the most frequent cause of diseases in children and the immunocompromised individuals. Hospital environments can act as reservoirs of multi-drug resistant pathogens, with the most contaminated site being the labour/delivery room followed by the dressing room and the operation theatre (Mathias et al., 2000; Solomon et al., 2017). Centre for Disease Control and Prevention (CDC, 2013) has documented increases in the number of nosocomial infections caused by antibiotic-resistant bacteria (ARB). Studies have also highlighted antibiotic resistance genes (ARGs), which can spread by adhering to airborne particles and their abundance in the indoor compared to outdoor sources such as soil water and sediments (Li and Yao, 2018). The bacterial contamination in operation theatres and paediatric wards has a major impact in terms of infection control in the hospital, health risk to the patients and medical staff, hence the need for this study. Airborne ARB and ARGs in hospital settings is poorly understood, with many studies focusing on other fomites particularly hospital equipment. Therefore, Airborne ARB and ARGs could pose serious human health issues especially in developing countries with poor infrastructure, poor infection control and lack of surveillance data on infectious agents.

An operating theatre is one of the hospital’s supposedly sterile facilities normally without windows, characterized by feature controlled temperature and humidity, where surgical operations are performed. It is usually separated from other hospital facilities, and accessed only by authorized personnel. The paediatric ward is the hospital’s facility assigned to provide health care for children under the age of 12. The operating theatre and paediatric wards were both selected in determination of the risk and possible transmission of opportunistic bacterial pathogens in highly immunocompromised surgery patients and children. This study was primarily focused on determining the occurrence, abundance, and relative diversity of bacteria in the indoor air of hospitals (paediatric and operating wards) in Botswana. The second objective was to further identify the antibiotic resistance patterns of airborne bacterial isolates by detecting the antibiotic resistance phenotypes and genotypes in selected bacteria. Furthermore, this research was extended to find correlation between the meteorological conditions (temperature and relative humidity) and the bacterial concentrations in the paediatric and operating wards.

MATERIALS AND METHODS

Description of study sites

The study was conducted at Palapye Primary Hospital (PPH), Letsholathebe II Memorial Hospital (LMH) and Nyangabwe Referral Hospital (NRH). PPH is located in Palapye town in the Central district, built in the 1970s and caters for the surrounding villages as it receives referrals from local and nearby clinics as well as rural health posts. LMH is one of the recently (opened in 2008) constructed hospitals in the town of Maun, Ngamiland district. LMH is located along the edges of the Okavango Delta in the region that has been previously hit by major southern African epidemics and
pneumonia; influenza, smallpox, bubonic plague, sleeping sickness, malaria, and bilharzia (Molefi, 2001, 2003; Mosothwane, 2015). NRH is located in the City of Francistown, North East district, built around 1989 is by far the largest hospital in the north eastern Botswana, and thereby receives most referrals from various regions across the country.

### Sample collection and isolation of airborne bacteria

Bacteria suspended in the indoor air of operating theatre wards and paediatric wards of the three hospitals were assessed. The air samples were collected between March 2016 and April 2017. In 2016, samples were collected in 2 seasons (summer and winter), and the other samples in 2017 (autumn). Botswana’s climate is characterized by four seasons; hot summers (November, December and January), wet autumns (February, March and April), cold and dry winters (May, June and July) and arid windy springs (August, September and October). Air samples were collected in duplicates once in a month, in the morning (9 am to 12 pm) and afternoon (2 to 5 pm) sessions.

The principle of air impaction was applied for the collection of airborne bacterial samples. Air was directed against the culture media plates using a portable Microbial Air Sampler MAS-100 NT® device (Merkmillpore, Merck KGaA Darmstadt, Germany), which is considered the gold standard among air samplers, at a flow rate of 100 L/min. The air sampler was placed 1.5 m above ground level within the hospital wards. Airborne bacteria were collected by impaction onto various selective and differential agar media for presumptive selective isolation of bacterial species; *Staphylococcus* species, *Brucella* species, *Pseudomonas* species, *Campylobacter* species, *Listeria* species, *Salmonella* species, and *E. coli* (Table 1). Cycloheximide (1 μg/μl, Sigma-Aldrich Co., St. Louis, MO, USA), previously shown not to affect bacterial counts was added to each media to inhibit growth of fungi (particularly saprophytic molds). Aerosolized bacteria were collected for 10 min on each of the 7-selective/differential media and non-selective nutrient agar media (for total airborne bacteria). Different selective media were used to target various potentially pathogenic bacterial species commonly associated with multi-drug resistance (Tapela and Rahube, 2019). After each sampling session, the culture media plates were incubated at appropriate aerobic/anaerobic and temperature conditions as shown in Table 1.

### Quantification and identification of airborne bacteria

To estimate airborne bacterial concentrations, the number of colony forming units growing in the respective media were counted and related to the volume of the air sampled. The MAS-100 NT® air sampler was set at an inflow rate of 100 L/min, the volume of air collected by air sampler per plate was estimated to 1000 L for 10 min. Bacteria growing on the selective/differential plates were counted and expressed as the average values of colony-forming units per cubic meter (CFU/m³). The actual microbial count per m³ of air was corrected and calculated using the integrated Feller conversion table (or FELLER’s statistical correction table) provided by MAS-100 NT® air sampler operating manual.

Bacteria were then identified by morphological procedures according to Bergeys Manual of Determinative Bacteriology (Holt et al., 1994). Morphological characterization of the airborne bacteria was carried out by examining colony size, shape, and cellular arrangement. Several colonies were randomly picked and further confirmed by Gram stain and biochemical tests. A total of 300 isolates were randomly selected, purified and stored at -80°C in nutrient broth with 50% glycerol solution prior to DNA extraction and antibiotic resistance testing.

### Antibiotic resistance testing

A total of 300 pure culture isolates were assayed for resistance against various antibiotics at clinical breakpoint concentrations; ampicillin (32 μg/ml), cephalosporin (32 μg/ml), penicillin (16 μg/ml), erythromycin (8 μg/ml), Sulfonylamine (512 μg/ml), meropenem (4 μg/ml), tetracycline (16 μg/ml), ciprofloxacin (4 μg/ml), streptomycin (30 μg/ml) and trimethoprim (16 μg/ml), similar to those reported as minimum inhibitory concentrations (MIC) for standard antibiotic susceptibility testing (Rahube et al., 2014a). These clinically relevant antibiotics were selected for identification of resistance phenotypes and genotypes in both Gram positive and Gram negative bacteria. Isolates were aseptically transferred using sterile
toothpicks or sterile disposable inoculation loops into respective 50 grid squared nutrient agar plates containing the respective antibiotic concentrations. The antibiotic resistance testing method used was previously described in a recent publication by Tapela and Rahube (2019).

DNA extraction and PCR detection of antibiotic resistance genes

DNA extraction was carried out using the method described by Neela et al. (2015) with some modifications. Briefly, 1.5 ml of the overnight-cultured bacterial cells were harvested by centrifugation at 13,000 rpm for 10 min; the pellet was resuspended in 600 µl of lysis buffer (50 mM Tris hydrochloride -50 mM EDTA, pH 8.0, 50 mM NaCl and 5% SDS). To re-suspend the pellet, and avoiding formation of foam, the tube was inverted 25 times and gentle pipetting was done. Followed by incubation at 37℃ for 30 min. Nucleic acid purification was done by adding 200 µl of phenol and chloroform/isoamyl alcohol, followed by addition of 200 µl of chloroform and vortexing for 20 s. The sample was then centrifuged at 13,000 rpm for 3 min to pellet the protein, and the supernatant was transferred into a new tube for nucleic acid precipitation. This was followed by adding 600 µl of isopropanol and precipitation of the DNA by centrifugation at 13,000 rpm for 30 min to obtain the DNA pellet. The DNA pellet was washed in 500 µl ice-cold ethanol (70%), and dried for 5 min.

The DNA was resuspended in 100 µl TE buffer and stored at -20℃ prior to PCR analysis. The antibiotic resistance genes were detected by PCR method using specific primers targeting resistance genes to different clinically important antibiotics: Sulfonamide (sul1 and sul2), tetracycline (tetA and tetB), erythromycin (ermA, ermB and ermC), macrolides (mhpA), streptomycin (strA and strB), quinolone (qnrA), trimethoprim (dfr1) and class 1 integron mobile element (intI1) were used to amplify the respective antibiotic resistance gene targets (Table 2).

The PCR amplification was performed with a PCR Thermal Cycler (Proflex PCR system, Applied Biosystems). The PCR mixture contained: 2 µl of template DNA, 12.5 µl premix (EmeraldAmp® PCR Master Mix, TAKARA BIO INC), 1.5 µl of each of the primers (forward and reverse) and 7.5 µl molecular grade water. The following thermocycler parameters were used: initial denaturation at 95℃ for 5 min followed by 30 cycles at 98℃, annealing for 40 s to 1 min (the annealing temperature varied among the primers, as indicated on Table 2), this was followed by extension at 72℃ for 1 min with a final elongation at 72℃ for 1 min. DNA of previously confirmed target antibiotic resistant isolates were used as PCR positive controls and a none-template reaction included as negative control. The PCR products were analyzed using gel electrophoresis on a 1.0% (w/v) agarose gel stained with ethidium bromide. Electrophoresis was run at a constant voltage of 60 V for about 1 h 30 min in a horizontal tank with 0.5X concentration of Tris-Borate-EDTA (TBE) buffer. After electrophoresis, the gels were visualized on a UV transilluminator.

Meteorological data and statistical analysis

Temperature (Tm) and relative humidity (RH) were recorded at each sampling site with a handheld Thermocron iButtons (Dallas Semiconductors, Model DS1920). Tm and RH were then reported as an average of 1 h 30 minutes sampling period at each sampling location. The means and standard deviations were analysed using Graphpad Prism software (version 7.0). Differences between different hospitals, sites (operating theatre and paediatric wards) were assessed using the analysis of variance (ANOVA) test. Results with a p-value less than or equal to 0.05 (p ≤ 0.05) were considered to be statistically significant.

RESULTS AND DISCUSSION

Occurrence and abundance of airborne bacteria in operating theatre and paediatric wards

The assessment of the total airborne bacteria in hospitals revealed higher abundance of bacteria in paediatric wards than in the operating theatres (p < 0.05). The highest bacterial concentration was observed at PPH paediatric ward: 7.9 ×10² CFU/m³, followed by LMH paediatric ward (6.4 ×10² CFU/m³), followed by NRH paediatric ward (5.8 ×10² CFU/m³). The highest bacterial concentration in operating theatre was also observed at PPH (3.7 ×10³ CFU/m³), which was statistically significant compared to NRH (2.7 ×10³ CFU/m³) and LMH (2.6 ×10³ CFU/m³) as shown in Figure 1.

There is a consistent difference in bacterial concentrations between operating theatre and paediatric wards among all the hospitals. The results are expected, and the higher bacterial concentrations in paediatric wards could have been greatly influenced by the nature and usage, including higher population of patients at the ward compared to those of the operating rooms. Several other factors such as lack of ventilation systems and depending on the outdoor air may have attributed to the vast difference in bacterial concentration between the two different hospital units. Quidesat et al. (2009), reported that microbial concentrations in the operation rooms were dependent on the hospital setup; operating theatre wards nearer to other hospital units had higher bacterial concentrations compared to those located away from the rest of the hospital units. The level of hygiene and practice of aseptic techniques, the dirty areas separate from the operating theatre rooms, the patient flow from arrival to discharge may also contribute to airborne bacterial concentrations. The high bacterial concentrations in PPH operating theatre ward could be attributed by the washrooms located adjacent to the entrance of the operating room as well as uncontrolled traffic and activities by health care workers around the areas in close proximity to the operating theatre. The PPH operating theatre ward had the highest airborne bacterial concentration compared to other operating theatres, PPH operating theatre is the hospital’s only major operating room, and therefore it experiences large volumes of activities on a daily basis. The PPH hospital is more than 30 years old, the design, layouts, furnishings, fittings, floor coverings and ventilation systems may have a significant impact on the cleaning of the unit consequently giving rise to the airborne bacterial contamination. In addition, PPH autoclave room that receives dirty materials and equipment from the operating room and the rest of the hospital is closer to the operating room thereby increasing the transfer of airborne bacteria to the operating room. Some studies have also reported that the sink drains are frequently colonized by large numbers of bacteria therefore serve as potential reservoir for aerosolized pathogens or opportunistic microorganisms.
Table 2. List of primer pairs used to amplify antibiotic resistance genes in this study.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Resistance phenotype</th>
<th>Primer pairs*</th>
<th>Primer sequence</th>
<th>Ann. temp (°C)</th>
<th>Size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>tetA</td>
<td>Tetracycline</td>
<td>tetA-L, tetA-R</td>
<td>GCCGGTCTTCTTCATCATGC, GCCGAGCGAGGCAAGTAGA</td>
<td>46.2</td>
<td>500</td>
<td>Boerlin et al. (2005)</td>
</tr>
<tr>
<td>tetB</td>
<td>Tetracycline</td>
<td>tetB-F, tetB-R</td>
<td>CATTAATAGGCATCGCTG, CATTAATAGGCGATCGCTG</td>
<td>50.5</td>
<td>930</td>
<td>Memon et al. (2016)</td>
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<tr>
<td>mphA</td>
<td>Macrolide</td>
<td>mph(A)-F, mph(A)-R</td>
<td>AACTGTACGACTTGC, GGTACTCTTCGTACC</td>
<td>50.0</td>
<td>837</td>
<td>Sutcliffe et al. (1996)</td>
</tr>
<tr>
<td>ermA</td>
<td>Erythromycin</td>
<td>ermA-F, ermA-R</td>
<td>GTTCAAGGACAAATACATTACAGAG, GGATCAGGAAAAGGACATTTC</td>
<td>58.0</td>
<td>421</td>
<td>Castro-Alarcon et al. (2011)</td>
</tr>
<tr>
<td>ermB</td>
<td>Erythromycin</td>
<td>ermB-F, ermB-R</td>
<td>AAAACCTACCGCCATACCA, TTTGCCGTTCATTGCTT</td>
<td>43.4</td>
<td>359</td>
<td>Castro-Alarcon et al. (2011)</td>
</tr>
<tr>
<td>ermC</td>
<td>Erythromycin</td>
<td>ermC-F, ermC-R</td>
<td>GAAATCGGCTCAGGAAAAGG, TAGCAAAACCCTATTTACAG</td>
<td>50.7</td>
<td>572</td>
<td>Castro-Alarcon et al. (2011)</td>
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<tr>
<td>qnrA</td>
<td>Quinolones</td>
<td>qnrA-F, qnrA-R</td>
<td>ATTTCTACGCGAGGATTGTCG, GATCGGCAAAGGTTAGGTCA</td>
<td>49.4</td>
<td>516</td>
<td>Robicsek et al. (2006)</td>
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<tr>
<td>dfr1</td>
<td>Trimethoprim</td>
<td>dfr-F, dfr-R</td>
<td>CCAACCGAAAGTATGCGCTCG, GTATCTACTTGATCGATCTT</td>
<td>45.6</td>
<td>171</td>
<td>Sunde (2005)</td>
</tr>
<tr>
<td>int1</td>
<td>Class1 Integrase</td>
<td>int1-F, int1-R</td>
<td>GCATCCTCGGTTCATTGC, GTGTGGCGCGGTCCATTGC</td>
<td>50.4</td>
<td>457</td>
<td>Yousefi et al. (2010)</td>
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<tr>
<td>strA</td>
<td>Streptomycin</td>
<td>strA-F, strA-R</td>
<td>CCTGGTGATACCGGACAATT, CCAATCGCAGTAGAAGGC</td>
<td>50.0</td>
<td>546</td>
<td>Boerlin et al. (2005)</td>
</tr>
<tr>
<td>strB</td>
<td>Streptomycin</td>
<td>strB-F, strB-R</td>
<td>ATCGTCAAGGATTGAAAAC, GGATCGTAGAACATATTTGC</td>
<td>46.9</td>
<td>500</td>
<td>Madsen et al. (2000)</td>
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<tr>
<td>sul1</td>
<td>Sulfamethoxazole</td>
<td>sul1-L, sul1-R</td>
<td>GTGACGGTTGTCGGATTTC, TCCGAAGGGTATTGCGCT</td>
<td>54.7</td>
<td>921</td>
<td>Lanz et al. (2003)</td>
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<tr>
<td>sul2</td>
<td>Sulfamethoxazole</td>
<td>sul2-L, sul2-R</td>
<td>CGGCGATCGTCACATACACT, TGTGCGGATGAGTCGCTC</td>
<td>51.5</td>
<td>721</td>
<td>Boerlin et al. (2005)</td>
</tr>
</tbody>
</table>

*F; Forward primer, R; reverse primer, Ann. Temp.; annealing temperature.
NRH operating theatre ward also had concerning abundance of airborne bacteria. This facility is also poorly located; the main entrance to the ward is along one of the busy corridors of the hospital and also opposite the Intensive Care Unit (ICU). Compared to PPH, NRH has a relatively better infrastructure; however, the ventilation system of most of the hospital units at NRH are malfunctioning, there have been reports from the NRH operating theatre wards that the air conditioning systems were for a long time in bad conditions (personal communication). The poor air ventilation and unstable indoor temperatures were experienced during sampling. The findings at LMH operating theatre ward were as expected, the bacterial concentration was at the lowest compared to the other hospitals’ operating theatre wards indicating a much cleaner indoor air, which could have been attributed to the cleanliness observed at the ward. PPH bacterial loads for both operating theatre and paediatric wards remains a major concern as revealed by a statistical difference when compared with the other two hospitals. Similar studies on the assessment of bacterial concentrations in hospital operating rooms such as at State Railway Hospital (Lublin, Poland) revealed that the airborne bacterial concentrations varied from 10 to $10^2$ CFU/m$^3$ (Augustowska and Dutkiewicz, 2006), which is in agreement with the findings of this study. The results are also consistent with other study findings elsewhere (McCarthy et al., 2000; Li and Hou, 2003), where microbial concentrations in operating rooms were notably lower compared to other hospital units.

The paediatric wards care for children less than 12 years. Those under the age of 7 years are usually accompanied by parents during hospitalization hence the higher population of the occupants, consequently this setup leads to increased inflow of airborne bacteria. In addition, products such as food, fruits and inanimate objects like toys from the external environment are frequently brought in by visitors into the paediatric wards and may contribute to the increase in airborne bacteria; these were documented as important source of hospital contamination (Quidesat et al., 2009). Similar to operating theatre wards results described previously, poor and deficient hygienic conditions due to minimal disinfection procedures across the three hospital’s paediatric wards might have given rise to the higher concentrations of observed airborne bacteria. The high abundance of airborne bacteria within the PPH paediatric ward might also be attributed to the age of the building. PPH paediatric ward is dimensionally too small to accommodate the inpatients, the ward is made up of a single room that is usually congested resulting in poor air circulation and also making it hard for cleaning. NRH paediatric ward also had high concentration of airborne bacteria, the ward has multiple rooms that are incompletely partitioned and separated from each other, hence allowing for free airflow between the rooms. There are no air conditioning systems at the NRH paediatric wards, therefore the ward depends on windows and doors for ventilation and this could lead to more inflow of airborne bacteria from the surrounding outdoor air. The paediatric ward of the LMH is ideally constructed, the rooms are well spaced making it easy for appropriate cleaning, the air conditioning system is in good condition, and also the ward is not congested. However, the results still reveal relatively high abundance of airborne bacteria.
compared to other paediatric wards. A study on airborne transmitted infections in hospitals revealed mean bacterial contamination values ranging between 3.0 - 7.0 \times 10^2 \text{ CFU/m}^3 during hours with less human activities and 6.0 \times 10^3 \text{ CFU/m}^3 during bed making hours (Woldu et al., 2013). Roberts et al. (2006) ascertains that influence of anthropogenic activities have an effect on the rise of airborne microbial concentrations in hospital rooms.

**Diversity and abundance of airborne bacterial species in operating theatre and paediatric wards**

The occurrence of diverse airborne bacterial species was observed in all three hospitals’ operating theatre and paediatric wards. In operating theatre wards, high concentration was recorded for *Pseudomonas* species; 1.3 \times 10^2 \text{ CFU/m}^3 (PPH), 1.2 \times 10^2 \text{ CFU/m}^3 (LMH and NRH), *Brucella* species; 7.4 \times 10^1 \text{ CFU/m}^3 (NRH), 4.4 \times 10^1 \text{ CFU/m}^3 (PPH), 2.7 \times 10^2 \text{ CFU/m}^3 (LMH), *Listeria* species, 1.3 \times 10^3 \text{ CFU/m}^3 (PPH), 3.8 \times 10^2 \text{ CFU/m}^3 (NRH) and *Staphylococcus* species; 3.3 \times 10^2 \text{ CFU/m}^3 (PPH), 2.4 \times 10^2 \text{ CFU/m}^3 (NRH), 2.0 \times 10^2 \text{ CFU/m}^3 (LMH). The least abundant bacteria were *E. coli*; 0.2 \times 10^3 \text{ CFU/m}^3 (LMH), 0.1 \times 10^3 \text{ CFU/m}^3 (PPH and NRH), and *Campylobacter* species 0.2 \times 10^2 \text{ CFU/m}^3 (NRH), 0.1 \times 10^2 \text{ CFU/m}^3 (LMH and PPH). *Salmonella* species were not detected in all three hospital operating theatre wards (Figure 2a).

Similarly in paediatric wards, the highest concentrations were recorded for *Pseudomonas* species; 2.8 \times 10^2 \text{ CFU/m}^3 (LMH), 2.7 \times 10^2 \text{ CFU/m}^3 (PPH), 1.8 \times 10^2 \text{ CFU/m}^3 (NRH), *Listeria* species; 1.7 \times 10^2 \text{ CFU/m}^3 (LMH), 1.2 \times 10^2 \text{ CFU/m}^3 (PPH), 2.1 \times 10^1 \text{ CFU/m}^3 (NRH), *Brucella* species; 1.8 \times 10^2 \text{ CFU/m}^3 (LMH), 1.0 \times 10^2 \text{ CFU/m}^3 (PPH), 5.3 \times 10^1 \text{ CFU/m}^3 (NRH) and *Staphylococcus* species; 1.4 \times 10^2 \text{ CFU/m}^3 (NRH), 7.1 \times 10^1 \text{ CFU/m}^3 (LMH). The least abundant bacteria were *Campylobacter* species; 1.1 \times 10^1 \text{ CFU/m}^3 (LMH), 0.9 \times 10^1 \text{ CFU/m}^3 (PPH), 0.6 \times 10^1 \text{ CFU/m}^3 (NRH). *E. coli* and *Salmonella* species at the lowest concentrations (0.1 \times 10^1 \text{ CFU/m}^3) in all paediatric wards (Figure 2b).

*Pseudomonas* species were the most abundant bacteria from all the operating theatre and paediatric wards. *Brucella, Listeria* and *Staphylococcus* species were also predominant. Findings from other studies where airborne bacteria were isolated in hospitals are in accordance with our results (Borrego et al., 2010; Qudiesat et al., 2009; Gilbert et al., 2010; Solomon et al., 2017). These studies also revealed higher prevalence of potentially pathogenic bacteria in the hospital air such as *Staphylococcus* species and *Streptococcus* species. *Pseudomonas* species, especially *P. aeruginosa* was also found to be prolific in the hospital atmosphere. *P. aeruginosa* is of particular importance due to its immense association with fatal nosocomial outbreaks (Hota et al., 2009; Gilbert et al., 2010). It is amongst the leading causes of nosocomial pneumonia associated with high mortality rates in healthcare settings (Krzowska-Firycz et al., 2014). In hospitals *P. aeruginosa* has been previously isolated from various environments especially around water source plumping systems such as sinks and drains (Prussin et al., 2015; Solomon et al., 2017). Hota et al. (2009) support these findings and further suggest that *P. aeruginosa* is aerosolized during hand washing thereby easily transmitted into the atmosphere. Other abundant bacteria genera in our study were *Listeria* species, these were also observed in similar studies conducted in hospitals (Sarica et al., 2002), where airborne *Listeria* among others were reported to be predominant in various areas of the hospital under study. *Listeria* species such as *Listeria monocytogenes* have been reported to be a health concern contributing to fatality rates of 20-30% among hospitalized patients (Swaminathan and Gerner-Smidt, 2007). These bacteria although they are foodborne, they are likely to enter the atmosphere from the hospitalized patients and/or their food. This is supported by the relatively higher concentration of the *Listeria* species in paediatric wards where food is allowed compared to operating theatre wards.

Another rather unusual bacterial occurrence to highlight is the *Brucella* species in all the three hospitals’ operating theatre and paediatric wards. Brucella is a genus of Gram negative bacterias of zoonotic origin. It’s occurrence in hospital units certainly warrants further investigation. The gastrointestinal bacteria which is also classified among foodborne pathogens, *Salmonella* species were only isolated from the paediatric wards in LMH and PPH at low concentrations. Similar to *Listeria, Salmonella* species isolated may also have entered the air from the contaminated food from the hospital kitchen or the food brought in by visitors, or aerosolized upon release during baby nappy changing of an infected child. The airborne *Salmonella* species, although at significantly low concentrations are likely to settle on uncovered drinking water and food resulting in contamination. *Salmonella* outbreaks have been reported in hospitals (mostly paediatric wards and nurseries) and nursing homes (CDC, 2013).

**Distribution of antibiotic resistance genes in bacterial species**

Bacterial species (n=50) that exhibited resistance to more than 7 tested antibiotics were selected and isolated. The DNA of the isolates was screened for presence of targeted ARGs. The frequency (% occurrence) of ARGs detected in the selected isolates were; *dfrA* (36%), *mphA* (26%), *ermC* (12%), *strB* (10%). In addition, genes encoding the class 1 integron mobile element *infI1* (16%) were also detected. Antibiotic resistance genes: *tetA*, *tetB*, *qnrA*, *strA*, *ermA*, *ermB*, *sul1* and *sul2* were not
Figure 2. Diversity and abundance of bacterial species in (a) operating theatres and (b) paediatric wards. (LMH-Letsholathebe II Memorial Hospital, NRH-Nyangabwe Referral Hospital, PPH-Palapye Primary Hospital).
detected in any of the bacterial isolates tested (Table 3). The gene that confer resistance to trimethoprim (dfr1) was detected in most of the bacterial isolates (35%). dfr genes were widespread amongst bacterial species; these were identified from *Staphylococcus* species (isolated from LMH operating theatre and paediatric ward) and *E. coli* (isolated from NRH paediatric ward), *Listeria* and *Pseudomonas* species (isolated from PPH, LMH operating theatres and paediatric wards). The high frequency of dfr genes in these environments is likely due to the wide application of trimethoprim in the treatment of urinary tract infections that are common in hospitalized patients. According to Jury et al. (2010), dfr genes are mostly associated with Class 1 integron mobile elements (conserved with sulfonamide, sul1 resistance gene) that can be horizontally co-transferred by plasmids, contributing to rapid spread of multiple resistance genes amongst bacterial communities.

In the current study, the presence of gene encoding macrolide 2'-phosphotransferase I, mphA gene was identified in 26% of the airborne bacterial species; *Staphylococcus*, *Pseudomonas* and *Listeria*. The bacterial isolates from which mphA gene was isolated were from NRH paediatric ward, LMH operating theatre ward and the paediatric ward. The findings of this study are in agreement with those from Nakamura et al. (2000) where mphA gene was identified in *P. aeruginosa* clinical isolates. mphA gene has also been detected in clinical isolates belonging to *Enterobacteriaceae* and *Staphylococcaceae* (Sutcliffe et al., 1996; Faccone et al., 2018). Other macrolide resistance genes, ermA and ermB, that confer resistance to erythromycin were not identified in any of the bacterial isolates; however the percentage of the airborne bacterial isolates harboring the ermC gene was 12%. The bacterial strains carrying the ermC belonged to the *Staphylococcus* and *Pseudomonas* species, isolated from LMH and NRH paediatric wards. This study detected genes conferring resistance to macrolides in most of the *Pseudomonas* species. Similar results were found in a study conducted by Türkyılmaz et al. (2010) where genes conferring resistance to macrolides like ermC were also detected in the majority of *Pseudomonas* species.

Class 1 integrons (intI1) were detected in 16% of the bacterial isolates; *Staphylococcus*, *Pseudomonas*, *Listeria* species and *E. coli* from all the three hospitals. The intI1 gene is highly associated with multiple antibiotic resistances and this gene also regulates the expression of some exogenous resistance genes such as sulfonamide resistance gene (sul1) that is commonly located adjacent to Class 1 integrons (Gillings et al., 2015). The findings of this study are similar to those by Shin et al. (2015), in which the Class 1 integron carrying multiple resistance genes was highly prevalent among Gram-negative bacteria. Class 1 integrons are commonly associated with genes conferring resistance to certain antibiotics including ampicillin, tetracycline, trimethoprim, chloramphenicol, kanamycin, gentamicin, and streptomycin (Rahube et al., 2014b) (Table 3).

### Temporal variation of airborne bacteria

Facilities like hospitals commonly have air-conditioning systems that facilitate temperature and humidity regulation thus the temperature and humidity recorded in hospital facilities are independent of the season. Figure 3 shows the findings of the effects of temperature and humidity on airborne bacterial concentration in the three hospitals under study.

Temperature and relative humidity (RH) are reported as an average of a 1 h 30 min sampling period for each sampling. During the study, temperature ranged between 20 and 29°C, RH ranged between 30 and 72%. The bacterial concentrations at PPH were relatively high in May 2016 (4.6 × 10^2 CFU/m^3) and March 2017 (4.2 × 10^2 CFU/m^3) compared to August (2.1 × 10^2 CFU/m^3). LMH recorded high bacterial concentrations in August (6.3 × 10^5 CFU/m^3) compared to May 2016 (5.5 × 10 CFU/m^3) and April 2017 (8.8 × 10 CFU/m^3). NRH also recorded the highest bacterial concentration of all in April (3.9 × 10^5 CFU/m^3), and low bacteria concentrations observed in June (9.9 × 10 CFU/m^3) and August (1.6 × 10^2 CFU/m^3) (Figure 3).

The results indicate that in addition to human activities discussed previously, the atmospheric bacterial concentration is directly affected by temperature. When temperatures are low (below 22°C) the bacterial concentration in the atmosphere is low then rises with the increase in temperatures (above 24°C). According to

<table>
<thead>
<tr>
<th>Bacterial</th>
<th>LMH</th>
<th>NRH</th>
<th>PPH</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus</em></td>
<td>dfr, ermC, intI1</td>
<td>mph(A)</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>dfr, ermC, intI1</td>
<td>mph(A), ermC, intI1</td>
<td>dfr, intI1</td>
</tr>
<tr>
<td><em>Listeria</em></td>
<td>dfr, intI1</td>
<td>mph(A)</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td></td>
<td>dfr, intI1</td>
</tr>
</tbody>
</table>

*; no ARG gene target was detected; LMH-Lesholathebe II Memorial Hospital, NRH-Nyangabwe Referral Hospital, PPH-Palapye Primary Hospital.

**Table 3. Distribution of ARGs in bacterial species isolated at the three hospitals units.**
Mouli et al. (2005) found that temperature is a significant factor for airborne bacteria, which governs the rate of change of water vapor and the rate of change of heat between the surface and environment. Other studies have also shown that the source of ventilation air in buildings; airflow rates, relative humidity and temperature correlate with the diversity and composition of indoor bacterial communities (So et al., 2017). The findings of this study also indicate a distinct trend between the effects of humidity and bacteria concentration observed at LMH and NRH. These results also reveal the distinctive spatial variation due to differences in the locations of the hospital units.

In hospital facilities, through the use of the air conditioning systems, the temperatures are usually maintained around 24°C. However, in instances where the air conditioning systems are malfunctioning, the temperature and relative humidity in the outdoors may have an impact on the indoor air. In some occasions during this study, some of the hospitals’ air conditioning systems were malfunctioning, thus affecting the desired temperature and humidity, consequently affecting the bacterial abundance. Guiamet et al. (2011) reported that air movements caused by ventilation could avoid high microbial concentrations in the air. Qudiesat et al. (2009) further supports the effects of ventilation on airborne bacteria by reporting that old and poor ventilation systems might serve as potential source of airborne bacteria in hospital units. Augustowska and Dutkiewicz (2006) studied the diurnal fluctuations of the airborne bacteria levels in pneumonological department ward of a hospital over a period of a year. Their study recorded the highest airborne microbial concentration during autumn whereas the lowest was observed during winter.

**Conclusions**

Occurrence and high abundance of airborne, potentially pathogenic and antibiotic resistant bacteria in hospitals’ operating theatres and paediatric wards indoor air poses a major concern. The results of this study provide evidence that indoor air of the hospitals’ units is a hotspot for antibiotic resistance determinants (ARB, ARGs, mobile genetic elements), which could be due to prolonged exposure to antibiotics and cross contamination caused by free movement of airborne bacteria from one source to another. The risk of antibiotic resistance dissemination is very high in both hospital units and can lead to acquired infections. Mobile genetic elements such as Class 1 integrons are important in accumulation of clinically relevant ARGs, their occurrence in opportunistic human pathogens such as *Pseudomonas* species may facilitate transmission of ARGs to other pathogenic bacteria such as *Staphylococcus* species and *E. coli*. There is urgent need to develop surveillance tools for monitoring the movement of airborne bacteria particularly in operating theatres and paediatric wards in order to mitigate possible spread of nosocomial infections. Improvement of infrastructures (for example new designs for hospital units, installation of new air conditioning systems) will significantly impact on the reduction of airborne bacterial levels.
systems) and good hygiene practices are very critical for effective control of airborne bacterial concentrations in the units.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

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Antibacterial, muscle relaxant, and hypnotic effects of seeds of *Peganum harmala* on mice

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*Peganum harmala* seed extract has been frequently reported to possess antibacterial potential through *in vivo* studies. *P. harmala* L. (Zygophyllaceae) is one of the most famous medicinal plants used in traditional medicine of Iraq. The harmaline, harmalol and harmine exerted many pharmacodynamic effects on the central nervous system: stimulation and depression depending on the dose. *P. harmala* indicates a great variety of pharmacological activities such as antimicrobial, antitumor, antiinflammatory and monoamine oxidase (MNO) inhibitory activities. The most important components of *P. harmala* seeds are harmaline, vasicinone and deoxyrinosine. The antibacterial effect of *P. harmala* was studied. The antibacterial activity of aqueous extracts was determined by agar well diffusion method. It inhibited the growth of *Escherichia coli* and *Staphylococcus aureus*. All animals injected with 100 mg/kg b. w of aqueous extract of *P. harmala* show myorelaxation or incoordination; so the animals dropped down from the wire 3 consecutive times in 60 s. Aqueous extract of *P. harmala* also induced muscle relaxation and prolonged the sleeping time induced by pentoparpitol. These data suggest that *P. harmala* extract could inhibit the growth of *S. aureus* and *E. coli* strain *in vitro* and this activity may contribute to its chemopreventive effect.

**Key words:** Antibacterial, muscle relaxant, hypnotic, *Peganum harmala*.

**INTRODUCTION**

Antibiotics at the present time are produced either synthetically or through microbial fermentation. The development of microbial resistance is one of the greatest public health problems. This problem has promoted a continual search for a new source of antimicrobial agents. Medicinal plants were the first medicines and have been used since ancient time (Seyyednejad and Motamed, 2010) and they continue to be used by various cultures around the world (Mahmoudian et al., 2002). All drugs from plants contain substance such as alkaloids, essential oils, phenols, unsaturated long chain aldehydes, peptides, ethanol, methanol, and butanol-soluble compounds with specific therapeutic activities (Servention et al., 1999). The antibacterial activities of several species of plant have been reported by many researchers (Cam, 2001; Sagdic and Ozcan; 2003). *Peganum harmala* is a perennial herbaceous, glabrous plant that can grow up to 30-100 cm and is distributed throughout the Middle East, North of Africa. This plant is famous for its antimicrobial effect, and is
traditionally used as disinfectant. Also alkaid from P. harmala has vasorelaxant, antihesporidian, anticancer, antinociceptive, antitumor and antineoplastic and antipirptozol effects (Arshad et al., 2008; Moghadam et al., 2010; Prashanth and John, 1999).

P. harmala is traditional medicinal plant that is used for many purposes, particularly in treating gastrointestinal problems. This plant has different varieties with different chemical constituents, some which have antimicrobial activity. Previous extraction and purification of Peganum harmala showed that this plant contained harmaline, harmol, harmitine, banisterines, peganeine, vasicinone and rosinicnone alkaloids as well as harmala or turkey resin and fatty oils (Hashim and Jamel, 1988). It is used as sedative in restless and agitated patients. It seeds are known to possess hypothermic and anti-oxidative properties (Rezvan et al., 2016; Moloudizargar et al., 2013).

Harmaline and harmine alkaloids exert antibacterial activity against a wide spectrum of bacteria. They have antifungal activity against many fungal species. They cause shrinkages of the protoplasm of the fungal cell (Al-Janabi, 1988; El-Kady et al., 1993). This result is in disagreement with Amin et al. (2014) who reported that ethanol extract of P. harmala has no effect on E. coli, only n-butanol; chloroform of P. harmala seed showed good antibacterial activity. On the other hand, harmaline, harmalol and harmine exert many pharmacodynamic effects on the central nervous system, ranging between stimulation and depression depending on the dose.

Aqueous extract of P. harmala causes motor dysfunction and is manifested by sluggish movement and unstable walking or loss of balance (Al Maliki and Elisha, 1985). Alkaloids of P. harmala are valued for their interesting chemistry, and pharmacological potential. They possess antitumor, antileshmanial, ant-HIV, antibacterial MAO- inhibition (Ramadhman et al., 2013). This study was designed to investigate the antibacterial and central nervous effects of P. harmala on mice.

MATERIALS AND METHODS

Plant materials

Fresh P. harmala were purchased from local market in Iraq and botanically authenticated by National Herbarium Botany Directorate.

Extraction of P. harmala

Dry seeds of P. harmala were grounded in coffee machines for 2-3 min. The powder was mixed with sufficient amount of distilled water, and shaken overnight at room temperature. The mixture was filtered, and the solvent was removed by incubation at 37°C. Distilled water was used to dissolve the dried residue to give the required concentration.

Microbial strains

Organisms were received from Department of Laboratory Clinical Science, College of Pharmacy. Al Mustansiriya University reconfirmed by gram staining and subculturing in appropriate selective media. The Gram positive bacterium was Staphylococcus aureus and Gram negative was Escherichia coli.

Preparation of standard culture inoculation of test organisms

Three or four isolated colonies were inoculated in the 2 ml nutrient broth and incubated till the growth in the broth was equivalent to Mac-Farland standard (0.5%).

Experimental no 1 antibacterial assay

Kirby- Bouar method was performed by Muller Hinton agar (Oxoid) (Nair et al., 2005) poured on disposable plates. Holes of 5 mm in diameter were made after solidification of the agar. E. coli and S. aureus were uniformly distributed on the surface of the agar. 0.4 ml of 5 and 10 mg/ml of P. harmala extract were placed in the holes. The plates were incubated at 37°C, and examined after 24 h for the presence of growth inhibition zones. Ampicillin of 10 mg was used as positive control, while distilled water was used as negative control.

Experimental design and procedure

The animals were randomly separated into two groups of Swiss albino mice weighing 21-23 g. They were housed in polypropylene cages and maintained under controlled temperature conditions in a 12 h light-dark cycle. They were given ad libitum access to commercially available mouse chow and water.

Studying muscle relaxant effect

Two groups of mice (6 mice each) were used. The first group was given 100 mg extract/kg.b.w intraperitoneally, while the second group was given distilled water by the same route as control. Muscle relaxation was determined by test delatraction. By this test, the mouse was hung by its forepaws on a thin wire placed over a bench. Normal mice pull themselves on the wire almost immediately with the aid of their hind paws. Failure of the mice to pull themselves or drop down three consecutive times in sixty second means they have myorelaxation and/or motor incoordination (Elisha et al., 1988).

Studying the effect of potentiation of pentobarbital sleeping

Two groups of mice zone varying between 2-15 were given 100 mg of P. harmala extract/kg and distilled water, respectively. Thirty minutes later, sodium pentobarbitol of 50 mg/kg was administered intraperitoneally. The animals were observed at intervals of minutes. They were placed on their backs and touched slightly with a glass rod. The period of losing righting reflex was taken as sleeping time (Al Maliki and Elisha, 1985; Clark, 1989).

Determination of the minimal inhibitory concentration

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by the macro broth dilution assay method (Mohamed, et al., 2010). A five-fold serial dilution of Mueller Hinton agar broth (50, 25, 12.5, 6.25, 3, 125) was used. The test organisms were incubated for 4 h to obtain concentration of 5 colony forming unit (CFU)/ml. Later, 50 micro of the inoculated
Table 1. Diameter of inhibition zone produced by the extract of *P. harmala*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Con.mg</th>
<th>S. aureus</th>
<th>E. coli</th>
</tr>
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<td>5</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3-15</td>
<td>3-12</td>
</tr>
<tr>
<td>Ampicillin</td>
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Table 2. Minimum inhibitory concentration (MIC) of *P. harmala* extract against *S. aureus*.

<table>
<thead>
<tr>
<th><em>P. harmala</em> concentration mg/ml</th>
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<th>2.5</th>
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<td>16.63</td>
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Table 3. Minimum inhibitory concentration (MIC) of *P. harmala* against *E. coli*.

<table>
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</tbody>
</table>

Table 4. Muscle relaxant effect of aqueous extracts of *P. harmala*.

<table>
<thead>
<tr>
<th>Group</th>
<th>Muscle relaxation</th>
<th>% of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>The isolation control</td>
<td>0/6</td>
<td>0</td>
</tr>
<tr>
<td><em>P. harmala</em></td>
<td>0/6</td>
<td>100</td>
</tr>
</tbody>
</table>

both was transferred into each dilution tube. Other dilution tubes were incubated at 37°C. The MIC was determined as the lowest concentration of *P. harmala*. It completely inhibited the growth of test organisms. The MBC was determined by culturing one standard drop of the tubes with no growth on Mueller Hinton broth and incubated at 37°C for 24 h. The control tubes were prepared with Gentamicin and free sterile solution of Muelle Hinton Broth.

Statistical analysis

Values reported are means ± SD. Results were statistically analyzed using the t-test, with P value less than 0.05 considered significant (Sorlie, 1995).

RESULTS

It was revealed that the extract of *P. harmala* (10 mg/ml) exhibited border spectrum as well as greater activity against *E. coli* and *S. aureus* with inhibition zone varying between 2-15 mm. Table 1 shows that the water extract of *P. harmala* exerted antibacterial activity against *E. coli* at 10 mg/ml; the mean of the inhibitory zone is 3-12 mm at 20 mg/ml. It exerted also antibacterial activity against *S. aureus*. The mean of the inhibitory zone is 2-9 mm. Table 2 shows the minimal inhibitory concentration of *P. harmala* against *S. aureus*. Table 3 shows the minimal inhibitory concentration of *P. harmala* extract against *E. coli*. Table 4 shows that all animals injected with the 100 mg/kg.b.w of aqueous extract of seeds of *P. harmala* showed myorelaxation or incoordination; so all the animals dropped down from the wire 3 consecutive times in 60 s. Table 5 shows that *P. harmala* prolonged the sleeping effect of pentobarbitol sodium.

DISCUSSION

The antibacterial activity of *P. harmala* may contribute to the flavonoid of dichloro. A biochemical analysis of *P. harmala* showed that this plant contains isoflavonoids with different side chains; flavonoids possess antibacterial activity against many bacterial species (Seyyednejad and Motamedi, 2010; Mahmoudian et al., 2002). *P. harmala* showed high antibacterial activity. This result is agreement with Arshad et al. (2008) and Moloudizargari et al. (2013) who found that *P. harmala* inhibits the growth of the tested bacteria. The inhibition produced by the plant extract against bacteria depends upon various extrinsic and intrinsic *S. aureus* and assigned as a source of antibacterial compounds against *S. aureus* and *E. coli*. Harmine has previously been identified as possessing antibacterial activities against several species of bacteria.
Shahverdi et al. (2005) who reported the significant activity of smoked dichloremethane extract of P. harmala seed against several species of Gram positive bacteria including Bacillus subtilis and harmine found it very effective against the Gram negative bacterium, Proteus vulgaris. This result is in agreement with Benbott et al. (2012) who report P. harmala is a potential source of antibacterial drug against various pathogenic bacteria, and disagreement with Mohamdeen et al. (2015) who report ethanol extract of P. harmala did not affect E. coli and n-butanol; and chloroform of P. harmala seed showed good antibacterial activity. This result is also in agreement with Fatma et al. (2016) who report that the flavonoids extract of P. harmala is useful to treat S. aureus. Muscle relaxant effect or incoordination caused by P. harmala are related to its motor dysfunction effect previously described by Al Maliky and Elisha (1985). The authors show that mice injected with aqueous extraction of P. harmala show sluggish movement, unstable walking or loss of balance. While hypnotic effect of P. harmala may be related to its activities on central nervous transmission via its interference with ionic exchange (Laks and Pruner, 1989). This result is in agreement with Mina et al. (2015) who report that P. harmala possesses various pharmacological activities such as analgesic. These results will encourage us to undertake further studies regarding the isolation and characterization of the active principle present in the active extract. Moreover clinical studies are required to understand the mechanisms along with the actual efficacy of these herbal extracts in treating various infectious.

**CONFLICT OF INTERESTS**

The author has not declared any conflict of interests.

**REFERENCES**


Hashim YM, Jamel M (1988). Medical plant between scientific research and popular medicine DarAuthor:77. Note: Not present on the online because the online begin in Iraq after 2003.


**Table 5. Hypnotic effects of aqueous extracts of P. harmala 100 mg/kg 30sec after 50 mg/kg of pentobarbital sodium.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Time of sleep/minute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.33±1.067</td>
</tr>
<tr>
<td>P. harmala</td>
<td>11.15±3.017*</td>
</tr>
</tbody>
</table>

*Significant P<0.05.