academicJournals

Vol. 12(1), pp. 19-26, 7 January, 2018
DOI: 10.5897/AJMR2017.8743
Article Number: 4FEB52655665
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

In vitro antibiotic resistance patterns of *Pseudomonas* spp. isolated from clinical samples of a hospital in Madinah, Saudi Arabia

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Received15 October, 2017; Accepted 30 November, 2017

***Pseudomonas* spp. are the leading cause of nosocomial infections. Rise in multidrug resistance among clinical isolates limit therapeutic options and hence increase mortality rate. Clinical samples (6840) from a hospital in Madinah, Saudi Arabia were collected for a duration of 14 months to study the frequency, antimicrobial sensitivity pattern and seasonal variations of *Pseudomonas* isolates. Conventional biochemical tests were done to identify the probable organism and antibiotic susceptibility was performed by disc diffusion method and Phoenix automated microbiology 100 ID/AST system. *Pseudomonas* represented 6.5% of all positive samples of which 65% were from males. Majority of the organisms (85%) were isolated from sputum and wound swabs followed by catheter tips (6.4%) and throat aspirates (3.4%). From the remaining samples, less than 1% organisms were obtained. Assessment of antimicrobial susceptibility to 11 different antibiotics revealed that imipenem was the most effective with highest sensitivity of 99.5%, and low intermediate resistance of only 0.5%. This was followed by ciprofloxacin (97.5%), ceftazidime (96.3%), cefpiramide/amikacin (94.1%), aztreonam (93.2%), gentamycin (87.7%), ampicillin (83%), and cotrimoxazole (80.1%). The most resistant drugs included augmentin (25%), cotrimoxazole (19.9%), ampicillin (17%) and gentamycin (12.3%) while the least resistant were ciprofloxacin (1.5%) and imipenem (0%). Results recommend imipenem as a promising antibiotic against *Pseudomonas* infections. In case of resistance to imipenem, ciprofloxacin, ceftazidime, cefpiramide, amikacin, and aztreonam may be recommended. In acute cases, *Pseudomonas* infections may require combined antimicrobial therapy. Frequency of these infections was the lowest (17%) during spring. It was the highest (30%) during summers and winters but reduced to 22% during autumn maybe due to better hygiene during pilgrimage season.**

Key words: Enterobacteriaceae, *Pseudomonas*, antibiotic resistance pattern, antimicrobial susceptibility, multi-drug resistance.

INTRODUCTION

Antibiotic drug resistance in pathogenic organisms is a universal problem now with severe treatment issues (Fair and Tor, 2014; Yezli et al., 2014; Chika et al., 2017). A gradual increase in drug resistance has been observed in most of the gram negative bacterial species, the main reason being excessive use and misuse of broad spectrum antibiotics (Fair and Tor, 2014; Ventola, 2015; Mahmoud *et* al., 2016). The use of antibiotics in animal feed stocks by the animal and food industry has also aggravated the condition (CDPC, 2013; Fair and Tor, 2014). Intensive Care Units (ICUs) are generally places where resistant pathogens flourish and are easily transmitted to other patients and healthcare workers (Chan, 2012; Aly and Balkhy, 2012). Gram negative bacteria account for upto 70% of nosocomial infections in ICUs (Peleg and Hooper, 2010) revealing a major change in the pattern of drug resistance around the world. The basic reason for escalation in antibiotic resistance has to be comprehended along with properly designed infection control programs and more stringent prescription guidelines.

Pseudomonas is a genus of ubiquitous non-fermentative gram negative bacteria, the species of which are metabolically diverse and hence are found in a wide variety of places especially in hospitals where bacteria grow easily in moist environment (Arora et al., 2011; Chika et al., 2017). Majority of *Pseudomonas* spp. develop resistance to penicillin and other related beta-lactam antibiotics (Shaikh et al., 2015). These opportunistic pathogens are host to several intrinsic and acquired resistance genes which they can also exchange with other gram negative bacteria (Juan Nicolau and Oliver, 2010). Hence, *Pseudomonas* is responsible for the occurrence and spread of several important carbapenemases (β-lactamases) (Kittinger et al., 2016). *P. aeruginosa* has high environmental tolerance, impermeable outer membrane, forms biofilms and has a several siderophores and pigments that allow it to evade the innate immune system (Fair and Tor, 2014). All these factors increase its resistance manifold fold making *Pseudomonas* infections complicated and life threatening.

Pseudomonas aeruginosa is one of the leading cause of nosocomial infections especially among patients admitted to ICUs. Other species like *P. putida* and *P. fluorescens* cause a variety of infections in clinical settings (Kittinger et al., 2016). Contaminated catheters and other medical devices transmit these pathogens causing bacteraemia, sepsis, pneumonia, urinary tract infections (UTIs), burn and wound infections, etc (Peleg and Hooper, 2010). According to reports broad-spectrum antibiotics like imipenem, ceftazidime, and amikacin have been recommended for treatments of infections caused by multi-drug resistant *P. aeruginosa* however, ciprofloxacin continues to be the oral drug of choice (Izadpanah and Khalili, 2015).

The annual cost associated with antimicrobial resistance was estimated to be $55 billion in US alone. On September 2016, the United Nations General Assembly organized a high-level meeting on antimicrobial resistance to address this global problem (U Nations, 2016). In Saudi Arabia, *P aeruginosa* has appeared as the most commonly isolated organisms in hospitals, causing 11% of all nosocomial infections; up to 31% of these are due to gram-negative organisms (Yezli et al., 2014; Khan and Faiz, 2016). Reports from Saudi Arabia claim that although more than half of the isolates of *P. aeruginosa* remain susceptible to carbapenems, quinolones, and aminoglycosides; multidrug resistance is on the rise at an alarming rate (Al-Agamy et al., 2012; Yezli et al., 2014).

Klebsiella pneumoniae carbapenemase (KPC), New Delhi Metallo-beta-lactamase (NDM) and (Verona Integron-Mediated Metallo-β-lactamase (VIM) are enzymes that break down carbapenems and make them ineffective. These enzymes have been reported in *Pseudomonas* (CDPC, 2016). There has been a significant surge in ESBL-related infection cases throughout the world (Shaikh et al., 2015). Emergence of infections caused by ESBL, MBL, MDR and PDR *P. aeruginosa* strains is alarming as they create serious health issues and place a huge liability of morbidity, mortality and health care cost on patients. *Pseudomonas* spp. belongs to the natural bacterial community in surface waters, they are clinically relevant, and changes in their natural resistance profiles indicate anthropogenic influence. Hence, there is an urgent need for persistent and cautious global surveillance for multidrug-resistant bacteria. This study, therefore, aimed at investigating resistances of *Pseudomonas* spp. to clinically important antibiotics and to evaluate the resistance pattern of this species isolated from clinical samples of a Saudi hospital.

MATERIALS AND METHODS

Sample collection

Different clinical samples such as sputum, wound swab, bile, tracheal aspirate (Tr. asp.), throat aspirate (Th. asp.), catheter Tip, pus, abdominal abscess (Abd. ab.), ear swab, peritoneal wound

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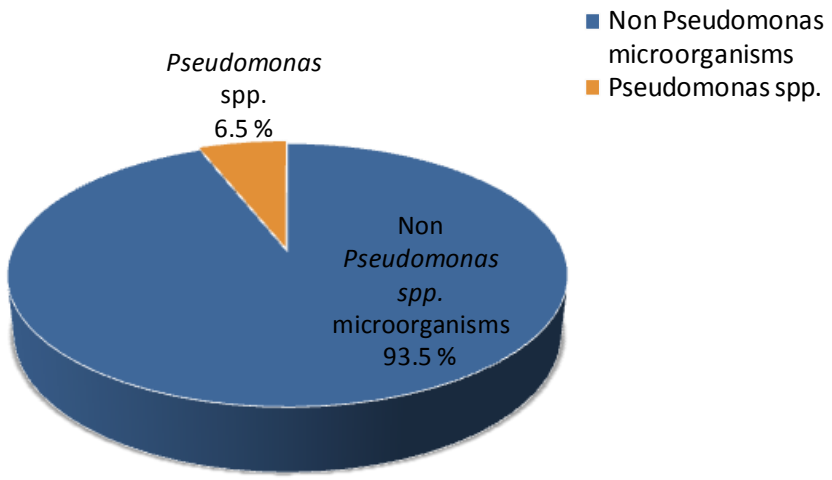

Figure 1. Percentage of *Pseudomonas* spp. in comparison to other clinical isolates.

swab (Peri. w.s.), pleural fluid (Pler. fluid), gall bladder aspirates (GB asp), necrotic tissue (NT), cystic fluid (CF) were collected from 6840 patients suspected of bacterial infection at King Fahd Hospital at Madinah, Saudi Arabia. Demographic data such as sex of the patients was recorded prior to sample collection.

Culture and Identification

The clinical samples were collected according to Centers for Disease Control and Prevention Specimen Collection Guidelines (CDPC, 2013), aseptically inoculated on plates of blood agar, chocolate agar, Cystine-lactose-electrolyte-deficient (CLED) agar and MacConkey agar (Oxoid Cambridge, UK) and incubated at 37°C for 24 h. Identification was done based on morphological characteristics of the colonies including size, shape, colour, pigmentation and haemolytic nature.

Biochemical characterization

Suspected *Pseudomonas* colonies isolated were further identified through biochemical tests using standard procedures (Barrow and Felthan, 2003) and Phoenix automated microbiology 100 ID/AST system (Becton Dickinson Company, Sparks, Md.). Identification included the following tests: nitrate reduction test, citrate utilization test, oxidase test, H_2S gas production, methyl-red test, indole test, urease test, Voges-Proskauer test and lactose fermentation (Forbes et al., 2007).

Antimicrobial susceptibility test

Susceptibility to antimicrobial agents was determined by using the disk diffusion method (Oqunshe, 2006), and Phoenix automated microbiology 100 ID/AST system (Becton Dickinson Company, Sparks, Md.). The following antimicrobial agents (obtained from BDH London, UK) were used: ampicillin (10 µg), augmentin [amoxycillin + clavulinic acid (20/10 µg)], gentamycin (10 µg), cotrimoxazole [Trimethoprim-Sulfamethoxazole 1:19 (25 µg)], amikacin (30 µg), ceftazidime (30 µg), aztreonam (30 µg), imipenem (10 µg), ciprofloxacin (5 µg), cefpiramide (30 µg) and piperacillin (100 µg). The inocula were prepared by growing the various *Pseudomonas* strains on separate agar plates and colonies from the plates were transferred with a loop into 3 ml of normal saline. The density of these suspensions was adjusted to 0.5 McFarland standards. The surface of Muller-Hinton agar (Oxoid Cambridge, UK) plate was evenly inoculated with the organisms using a sterile swab. The swab was dipped into the suspension and pressed against the side of the test tube to remove excess fluid. The wet swab was then used to inoculate the Muller-Hinton agar by evenly streaking across the surface. By means of a Disc Dispenser (Oxoid Cambridge, UK), the antibiotic discs were applied onto the surface of the inoculated agar and the plates were incubated overnight at 37°C. The diameter of zone of growth inhibition observed was measured and compared to the chart provided by Clinical and Laboratory Standards Institute (CLSI, 2015).

RESULTS AND DISCUSSION

Many studies have been done till now to estimate the antibiotic resistance pattern in ICUs around the world but unfortunately only a few studies have been reported from Saudi Arabia (Rotimi et al., 1998; Johani et al., 2010). The present work investigates the antimicrobial resistance pattern of *Pseudomonas* spp. isolated from patients of King Fahad Hospital, Madinah, one of the two holy cities visited by lakhs of pilgrims the whole year around. Clinical samples (6840) were collected from patients suspected of bacterial infection during a span of 14 months and screened for the gram negative bacteria. Results showed that of all the isolates screened, only 6.5% were *Pseudomonas* spp. (Figure 1). Isolates could not be recovered from some samples like urine, blood, ascetic fluid, nasal swabs, axilla, and perineum. It was observed that of the positive *Pseudomonas* isolates, 65% were from males while 35% were from females (Figure 2) indicating that males show greater vulnerability for these infections. *Pseudomonas* species were positive for oxidase test, catalase test, nitrate reduction test; citrate utilization test, gelatin hydrolysis, and motility; and

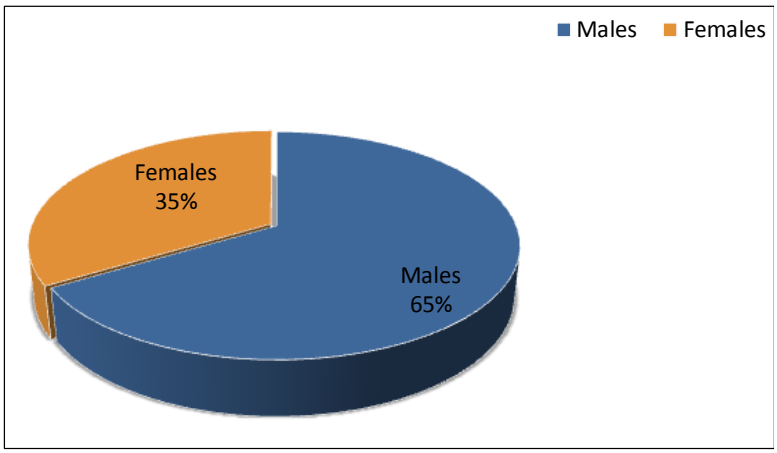

Figure 2. Percentage *Pseudomonas* spp. isolates from males and females.

negative for urease test, Voges-Proskauer test, H_2S gas production, methyl-red test, indole test, and coagulase test (Forbes et al., 2007).

Table 1 gives an estimation of the number of male and female samples isolated from different sources. Majority of the *Pseudomonas* species (85%) were isolated from sputum and wound swab samples of which 66.8% came from the sputum sample of male patients. Similarly, in case of wound swabs, 61.9% were from males and 38.1% were from females. A higher isolation rate from sputum and wounds has been reported in earlier studies as well (Ahmed, 2016; Golia et al., 2016). Samples from catheter tips and throat aspirates revealed greater *Pseudomonas* species from males. The percentage male to female ratio was 82.1:17.9 and 66.7:33.3 in case of catheter tips and throat aspirates, respectively. As reported earlier (Babay, 2007; Magliano et al., 2012), in case of wound swabs, the male to female ratio was 86:53 indicating that adult males are more susceptible to infection than adult females. A similar situation was observed with catheter tips and throat aspirates where male to female ratio of isolated specimens were 23:5 and 10:5, respectively. Results are not unexpected as in Saudi Arabia males represent a larger labor force and hence are exposed more to infections, pollution of all kind and accidents on roads and factories.

Only 1 sample each was available from ear, gall bladder aspirate, necrotic tissue and cystic fluid and that also from females. Since the number of samples is low, any substantial conclusion could not be drawn from these samples. One sample each was obtained from bile, abdominal abscess, and bed sores that is the male to female ratio in these three cases was 1:1. Peritoneal wound swab and pleural fluid provided 2 specimens each but only from males. Five specimens were isolated from tracheal aspirates where 40% were from males and 60% from females while four specimens were isolated from pus swabs where 75% were obtained from males and rest from females.

Figure 3 shows the percentage samples from various clinical sources that were positive for *Pseudomonas* species. As mentioned earlier, the majority of isolates were from sputum and wound swabs, 53.4 and 31.6%, respectively followed by catheter tips (6.4%) and throat aspirates (3.4%). Very few strains were obtained from other clinical sources. The percentage of *Pseudomonas* isolates from tracheal aspirates was 1.93% while from bile, abdominal abscess, peritoneal wound swabs, bed sores and pleural fluid it was 0.45%. GB aspirates, ear swabs, NT, and CF were poor sources providing only 0.23% positive isolates each. The percentage of other samples was not adequate enough to assess gender profile and give reproducible results. Earlier reports also state (Janda and Abbott, 2006; Babay, 2007; Magliano et al., 2012) that males are more vulnerable to infection than females, especially respiratory tract infections. These findings may be explained on the basis of their different anatomy, lifestyle, and socioeconomic conditions.

Antimicrobial drug sensitivity was performed by disc diffusion assay using antibiotic discs of ampicillin, augmentin, gentamycin, cotrimoxazole, amikacin, ceftazidime, aztreonam, piperacillin, imipenem, ciprofloxacin, cefpiramide. As shown in Table 2, imipenem was the most effective antibiotic against *Pseudomonas* species with 99.5% sensitivity followed by ciprofloxacin with 97.5% sensitivity. The high sensitivity of imipenem against gram-negative bacteria has been shown by others as well (Mokaddas and Sanyal, 1999; El-Tahawy, 2000; Bahashwan and Shafey, 2013; Dash *et* al., 2014). Ceftazine showed a sensitivity of 96.3%. Cefpiramide is a third generation cephalosporin antibiotic while amikacin is a fourth generation aminoglycoside. Both showed fairly good sensitivity of 94.1%; results

Table 1. Gender wise distribution of *Pseudomonas* specimens isolated from different sources.

Sex	Source of specimens														
	Sp	WS	Bile	Tr	Th	Cath	Pus	Abd	Ear	Peri	Pler	G B asp	Bed Sores	NT	CF
M	157 (66.8)	86 (61.9)	1(50)	2 (40)	10 (66.7)	23 (82.1)	3 (75)	1 (50)	0 (0)	2 (100)	2 (100)	0 (0)	1 (0)	0 (0)	0 (0)
F	78 (33.2)	53 (38.1)	1 (50)	3 (60)	5 (33.3)	5 (17.9)	1 (25)	1 (50)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)
Total	235	139	2	5	15	28	4	2	1	2	2	1	2	1	1

M, Males; F, Females; Sp, Sputum; WS, Wound swab; Tr, Tracheal aspirate; Th, Throat aspirate; Cath, Catheter Tip; Abd, Abdominal abscess; Peri, peritoneal wound swab; Pler, Pleural fluid; GB asp, Gall bladder aspirates; NT, Necrotic tissue; CF, Cystic fluid. Percentage (%) values are given in parentheses.

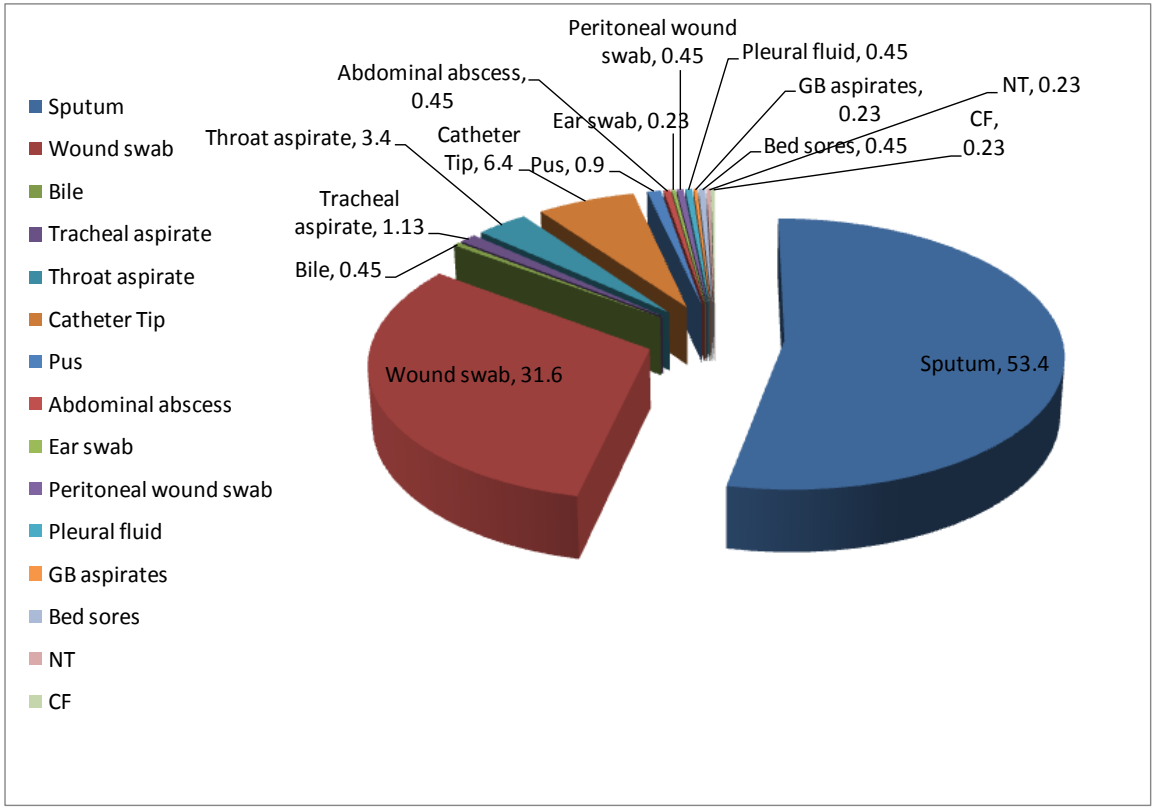

Figure 3. Percentage of *Pseudomonas* spp. specimens isolated from different clinical samples.

Table 2. Percentage (%) antimicrobial sensitivity pattern of *Pseudomonas* isolates to different antibiotics.

Antibiotic	Sensitive	Resistant	Intermediate
Ampicillin	83.0	17.0	0
Augmentin	75.0	25.0	0
Gentamycin	87.7	12.3	0
Cotrimoxazole	80.1	19.9	0
Amikacin	94.1	5.6	0.3
Ceftazidime	96.3	3.2	0.5
Aztreonam	93.2	5.6	1.2
Imipenem	99.5	0.0	0.5
Ciprofloxacin	97.5	1.5	1.0
Cefpiramide	94.1	5.3	0.6
Piperacillin	90.0	4.8	5.2

Table 3. Percentage (%) of *Pseudomonas* infection during different seasons.

Season	Percentage (%) of *Pseudomonas* infections
Summer (22 June -22 September)	30.1
Autumn (23 September -21 December): Pilgrimage season	22.1
Winter (22 December -30 Mars)	31.0
Spring (21 Mars-21 June)	16.8

being consistent with the previous studies (Dash et al., 2015; Ahmed, 2016).

The percentage sensitivity with the other antibiotics in the present study was in the sequence: aztreonam (93.2%) > piperacillin (90%) > gentamycin (87.7%) > ampicillin (83%) > cotrimoxazole (80.1%) > augmentin (75%). *Pseudomonas* used in the present study showed highest resistance to augmentin (25%) followed by cotrimoxazole (19.9%), ampicillin (17%), gentamycin (12.3%), amikacin (5.6%), aztrenam (5.6%), cefpiramide (5.3%), piperacillin (4.8) and ceftazidime (3.2%).

Bacterial infections have seasonal trends depending on several factors. Investigating their prevalence will assist in identifying specific risk factors that will help in improving and formulating new infection control strategies. Several reports claim that bacterial infections always peak during summers and winters (Eber et al., 2011; Psoter et al., 2013). Table 3 depicts the percentage of *Pseudomonas* infection during the four different seasons. It was observed that the percentage of infections was the highest during summers and winters (30 to 31%) followed by autumn (22.1%). During spring, the percentage was not too high being only 16.8%.

Winters are known for the outbreaks of bacterial infections especially related to the respiratory systems (Eber et al., 2011; Psoter et al., 2013). The autumn season incidentally coincides with the major pilgrimage period when a large number of pilgrims visit Madinah during Haj. But amusingly, the proportion of infection lowered in this period in comparison to the peak seasons (summers and winters). Increased proportions of infection during summers and autumn have also been reported earlier also (Psoter et al., 2013). This can be explained as during annual pilgrimage (Haj), the health authorities take special care to control outbreak of bacterial and other infections. Almost similar patterns were seen in case of *Proteus* and *Klebsiella* infections during the same period of study (Bahashwan and Shafey, 2013; Ghanem et al., 2017). Even though Makkah and Madinah expect huge influx of pilgrims throughout the year, it is during the Haj season that there are dangers of an epidemic outbreak. To circumvent the spread of infection, special precautions are taken which could be the reason for such low percentage of infection during the pilgrimage season. Saudi Arabia has taken a good initiative in reducing spread of resistant pathogens in healthcare units by implementing the World Health Organization (WHO) hand hygiene program and the Gulf Cooperation Council (GCC) Infection Control Program (Yezli et al., 2014). There is a need to introduce more such programs to control multidrug resistance in gram negative bacteria.

Conclusion

It may be concluded that males are at a greater risk of

Pseudomonas infections in comparison to females. Imipenem is the most effective antibiotic and can be prescribed to patients without any hesitation as it has the highest sensitivity and lowest resistance in this case. A larger number of *Pseudomonas* strains were found resistant to augmentin, cotrimazole and ampicillin suggesting that these antibiotics should be prescribed but with care. Summers and winters both seemed to have the highest infection percentage followed by autumn. Lack of awareness, self-medication and misuse of antibiotics has aggravated multidrug resistance in microbes. Compliance to infection prevention guidelines are essential to eliminate major outbreaks in the future.There is an ardent need to formulate and adhere to new guidelines for drugs based on their sensitivity profiles. Studies like these can help in developing rationalized local databases concerning antimicrobial resistance patterns, and hence formulate better infection control strategies in Saudi Arabia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

Authors would like to thank the Microbiology staff of King Fahd Hospital, Al Madinah Al Munawarah, KSA for their assistance with data collection.

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