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Frequency and Antimicrobial Susceptibility Pattern of *Pseudomonas Aeruginosa* in Human Pus Samples at Holy Family Hospital Rawalpindi

Ali MM[1], Mansoor R[2], Zahra QA[3], Liangliang L[4], Gangguo W[5], Ali R[3], Kazmi A[6]* and Khan MI[3]

[1]Pathology Department, Rawalpindi Medical University, Pakistan; ORCiD: 0000-0001-9755-6694

[2]Pathology Department, Rawalpindi Medical University, Pakistan

[3]Centers for Biomedical Engineering, University of Science and Technology of China, China;

[4]Centers for Biomedical Engineering, University of Science and Technology of China, China; ORCID: 0000-0003-3285-9947

[5]Centers for Biomedical Engineering, University of Science and Technology of China, China; ORCID:0000-0001-9596-3384

[6]Pathology Department, Al-Nafees Medical College and Hospital, Isra University, Pakistan

***Corresponding author:** Kazmi A, Pathology Department, Al-Nafees Medical College and Hospital, Isra University, Pakistan; ORCID: 0000-0001-6898-4630; Email: ahsankazmi101@gmail.com

Research Article

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Abstract

Background: Nosocomial infections are great threat for hospitalized patients and *Pseudomonas aeruginosa* has emerged as one of the most potent nosocomial pathogens along with its diverse mechanisms to counter the various antimicrobial agents such as aminoglycosides, fluoroquinolones, monobactems, third generation cephalosporins, carbapenams and broad-spectrum penicillins. *P. aeruginosa* is one of the well-known pyogenic bacteria and is 3rd leading cause of pyogenic infections with the variable frequency depending on geographical region and clinical setting. *P. aeruginosa* is intimately associated with pyogenic nosocomial infections.

Objectives: Since multidrug resistant strains of *P. aeruginosa* have posed serious threats and are frequently implicated in nosocomial infections.

Methods: Pus swab were sampled under aseptic conditions and cultured on blood and Muller Hinton agar. Gram reaction, pigment production, Oxidase, indole reaction and citrate test were used to confirm isolate. Antibiotic susceptibility was performed b Kirby Bauer technique.

Results compiled by us in this cross sectional study, showed 58 cases of *P. aeruginosa* out of 289 cases. This included 43% males and 57% females. Majority of the patients were of young age, with mean age 38 years. Antibiotic sensitivity revealed resistance to gentamicin was 50%, amikacin was 64%, ciprofloxacin and Aztronem 66%, Cefaparazone 69%, Tzaocin 71% and meropenem and sulzone was 79%. While Colistin and Ceftazidime were the most effective in 85% and 89% of cases respectively. The multidrug resistant strains of *P. aeruginosa* infections accounted for 32.76% of total *P. aeruginosa* infections. This study reveals high prevalence of multidrug resistant organisms at the set of our study. Based on this study, we suggest adopting the strategies to minimize the risk of nosocomial infections to slow down the rapidly growing multidrug resistance. These strategies may include, stricter antiseptic measures, fastening the recovery process and reducing the hospital stay and considering other alternates. Besides this, we would like to suggest the precise use of antibiotic susceptibility facility to reduce the nosocomial infection associated complications.

Keywords: *Pseudomonas Aeruginosa*; Antibiotic Susceptibility; Frequency; Antibiotic Resistance; Sensitivity Patterns

Introduction

Pseudomonas aeruginosa (P. aeruginosa) is a Gram-negative rod, is a part of our non-glucose fermenting normal microbial flora and widely distributed in natural environments (such as water and soil) [1]. It resides as an opportunistic pathogen, mostly involved in nosocomial infections in the immunocompromised patients [2]. *P. aeruginosa* is one of the well-known pyogenic bacteria and it is one of the leading causes of pyogenic infections with the variable frequency depending on geographical region and clinical setting. *P. aeruginosa* is intimately associated with pyogenic nosocomial infections as it is commonly found in hospital instruments [3]. The pyogenic infections caused by *P. aeruginosa* are characterized by the production of green-blue, foul smelling pus [4]. In the United States, every year 51,000 bacterial infections and 440 deaths are attributed to *P. aeruginosa* infections [5].

With the passage of time, *P. aeruginosa* has emerged as one of the most potent nosocomial pathogens along with its diverse mechanisms to counter the various antimicrobial agents such as aminoglycosides, fluoroquinolones, monobactems, 3rd generation cephalosporins, carbapenams and broad-spectrum penicillins [6]. The resistance to the above-mentioned antibiotics is attributed to low permeability of cell wall, production of inducible cephalosporinase, an active efflux and poor activity for target [7]. Studies suggest that the susceptibility pattern of *P. aeruginosa* depends on the site/specimen from which it is isolated. The multidrug resistance *P. aeruginosa* predominates in pus specimens [8]. The multidrug resistant *P. aeruginosa* infections account for 13% of total *P. aeruginosa* infections [5].

Since multidrug resistant strains of *P. aeruginosa* have posed serious threats and are frequently implicated in nosocomial infections. Risk factors for multidrug resistant *P. aeruginosa* infection include prolonged hospitalization, exposure to antimicrobial therapy and immunocompromised states. The increasing frequency of multidrug resistant strains has been the cause of concern for not only clinicians but also for microbiologists [9]. In the field of Microbiology, it is necessary to periodically assess the pathogenic characteristics/properties of microorganisms in order to keep an eye on the arsenal of resistance mechanisms they possess. Antimicrobial sensitivity testing is the cornerstone in the treatment of bacterial infections. With the passage of time, bacteria have developed resistance to various antimicrobial agents which has resulted in greater mortality and morbidity associated with infectious diseases. The present study is aimed to find out the frequency and antimicrobial susceptibility pattern of *P. aeruginosa* isolated from pus specimens. The study would enable physicians and microbiologists to detect the trends in the susceptibility profile of *P. aeruginosa* to commonly prescribe antipseudomonal antibiotics.

Materials and Methods

The study was a cross-sectional observational study carried out at the Department of Microbiology, Holy family Hospital, Rawalpindi which is a tertiary care hospital between April and December 2020. Two hundred and eighty-nine patients suspected with pyogenic infections were included in the study population irrespective of their age and gender. All pus specimens/swabs were collected passible aseptic conditions, irrespective of oozing site. Non-probability convenient sampling technique was used for sampling. Study was concluded after ethical approval from the ethical committee of Rawalpindi Medical University and Allied Hospitals Rawalpindi. For the identification of isolates, pus specimens were cultured on Blood agar and MacConkey's agar plates and incubated aerobically at 37˚C for 24 to 48 hours. The plates were observed after overnight incubation for growth which was identified by colony morphology, Gram staining and biochemical characteristics. In-addition isolates were also identified by commercially available API 20NE (BioMerieux, France). Moreover, isolates were identified as *Pseudomonas aeruginosa* on the basis of colony morphology, Gram staining, oxidase positivity, nitrate reduction, positive catalase test, production of pyocyanin pigment, citrate utilization and ability to grow at 42˚C.

The inoculum for susceptibility testing was prepared by touching the top of colonies of isolates with sterile wire loop and suspended in 0.5ml of broth (Equal to 0.5 McFarland standards). The susceptibility testing was performed by Modified Kirby Bauer disc diffusion method on Muller Hinton agar as per previously reported criteria [10,11]. The antibiotic disks were purchased from Oxoid, England. The surface of Muller Hinton agar plate was inoculated by streaking the plate with swab moistened with inoculum. The antibiotic disks were placed on the surface of streaked Mueller Hinton agar plate. The disks were placed about 15 mm away from the edge of the plate and 24 mm from each other.

The antibiotic disks used were; gentamicin (10µg), amikacin (30µg), ciprofloxacin (5µg), aztreonam (30µg), ceftazidime (30µg), cefoperazone (75µg), tazocin (10µg), meropenam (10µg), colistin (10µg) and sulzone (105µg). The plates were incubated at 37˚C for 24 hours. The plates were examined and zone sizes were measured with ruler after overnight incubation. *P. aeruginosa* American type control culture (ATCC) 27853 was used as quality control for susceptibility testing. The numerical data was entered in SPSS (version 20) software. Coding of variables was done to facilitate data analysis (Figure 1 & Table 1).

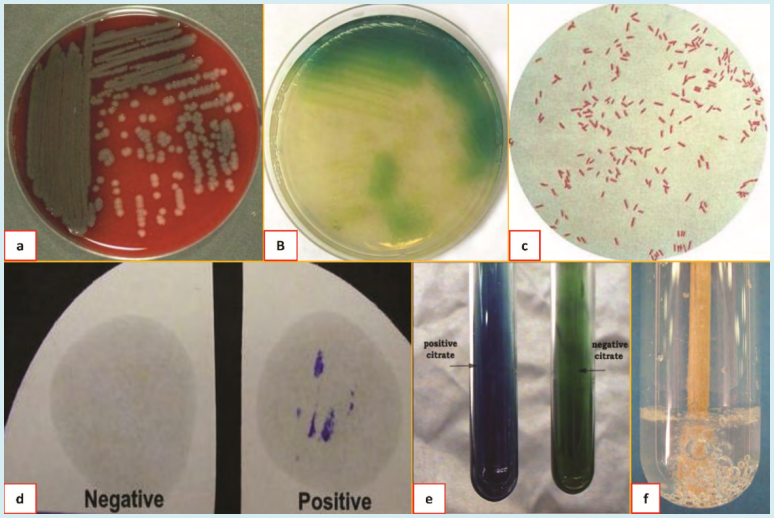

Figure 1: Colonies of *P. aeruginosa* on blood agar; b: Production of pigment on Muller Hinton agar by *P. aeruginosa*; c: Gram-negative rods of *P. aeruginosa* seen under microscope; d: Oxidase reaction; e: Citrate utilization test; f: Positive catalase test reaction.

Antibiotic Disk	Disk Content (In µg)	Diameter of zone (in millimeters)		
		Sensitive (S)	Intermediate (I)	Resistant (R)
Gentamicin	10	≥15	13-14	≤12
Amikacin	30	≥17	15-16	≤14
Ciprofloxacin	5	≥21	16-20	≤15
Aztreonam	30	≥22	16-21	≤15
Ceftazidime	30	≥18	15-17	≤14
Cefoperazone	75	≥21	16-20	≤15
Tazocin	10	≤21	18-20	≤17
Colistin	10	≥11	_	≤10
Meropenam	10	≥19	16-18	≤15
Sulzone	105	≥21	20-18	≤17

Table 1: Kirby Bauer Disk Diffusion Technique, Zone Diameter According to CLSI Guidelines.

Results

Frequency of *P. Aeruginosa* in Pus Specimens

Out of 289 positive pus samples, 58 were identified as *P. aeruginosa* with the frequency of 20% (58 x 100/289 = 20%) (Figure 2a).

Gender-wise Distribution

Gender-wise distribution showed that out of 58 isolates of *P. aeruginosa,* 33 (57%) were from males and 25 (43%) from females (Figure 2b).

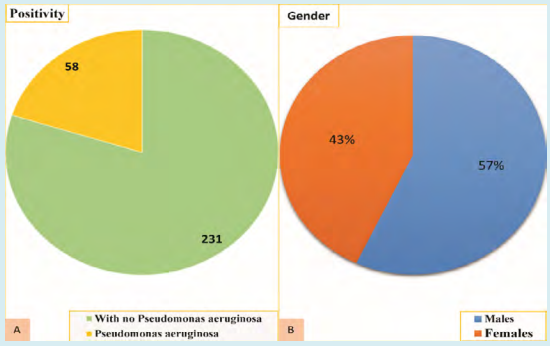

Figure 2: Positivity rates and genders contribution. A; prevalence of *P. aeruginosa* in total samples and B; shows gender distribution of patients.

Age-wise Distribution

The age distribution of patients ranged from 1 year to 80 years with mean age of approximately 38 years and standard deviation of 22.28. Patients belonging to the age group ≥ 50 years showed highest incidence of *P. aeruginosa* infections followed by age group ≤ 10 years (Figure 3).

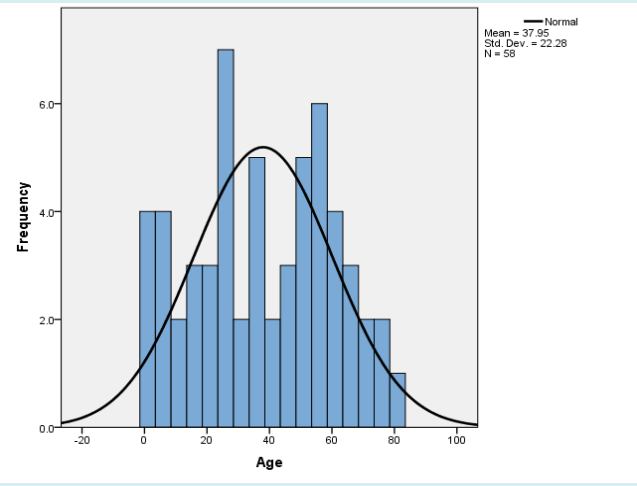

Figure 3: Histogram showing age distribution of patients.

Antimicrobial Susceptibility Profile of *P. Aeruginosa* Isolated from Pus Specimens

Antimicrobial sensitivity testing of *P. aeruginosa* isolates revealed that colistin and ceftazidime are the most potent antipseudomonal antibiotics. About 88% isolates were found to be susceptible to colistin and 85% to ceftazidime. Meropenam and sulzone also showed considerable antipseudomonal activity as 79% isolates were susceptible to each antimicrobial agent. Among the ten antimicrobial agents used in study, gentamicin showed poorest activity with only 50% isolates being susceptible to it. 66% isolates showed susceptibility to ciprofloxacin and aztreonam and tazocin showed antipseudomonal activity against 71% isolates and cefoperazone to 69% isolates. (Figure 4 & 5) Out of 58 isolates of *P. aeruginosa*, 19 were multidrug resistant strains. According to CDC, strain of *P. aeruginosa* is classified as multidrug resistant if it is simultaneously resistant to at least three classes of antimicrobial agents including beta-lactams, aminoglycosides, fluoroquinolones, monobactems and polymyxins (Table 2).

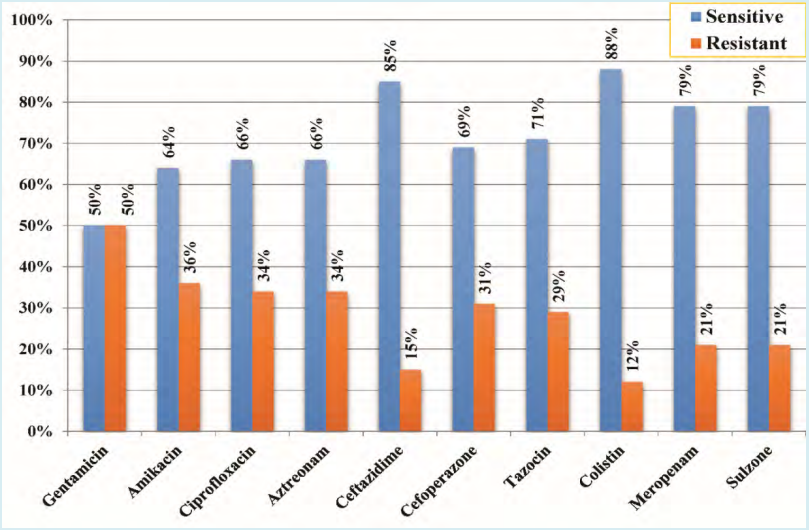

Figure 4: In-vitro susceptibility pattern of *P. aeruginosa* isolated from pus specimens to different antimicrobials.

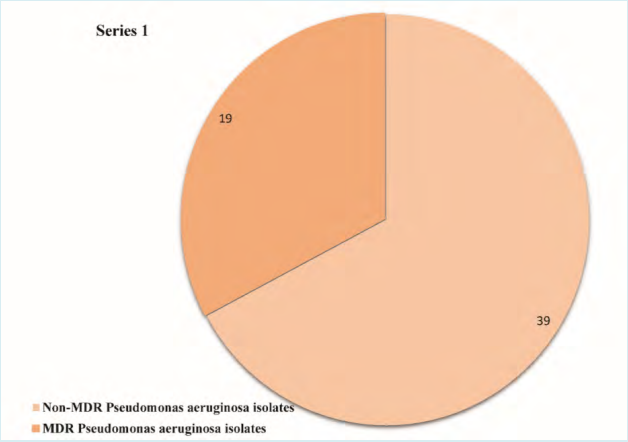

Figure 5: Prevalence of multidrug resistant *P. aeruginosa* patients infected with *P. aeruginosa.*

Antibiotic	Sensitive (%age)	Resistant (%age)
Gentamicin	50%	50%
Amikacin	64%	36%
Ciprofloxacin	66%	34%
Aztreonam	66%	34%
Ceftazidime	85%	15%
Cefoperazone	69%	31%
Tazocin	71%	29%
Colistin	88%	12%
Meropenam	79%	21%
Sulzone	79%	21%

Table 2: Showing susceptibility profile of *P. aeruginosa* isolated from pus specimens.

Discussion

Treatment of infection diseases are a great challenge for medical sciences during this evolving state of infectious agents. Very often, infectious diseases are associated with mortal/morbid complication or disabilities [12,13]. *P. aeruginosa* is common in the environment, which belongs to the *Pseudomonadaceae* family. It remains to be a major opportunistic pathogen especially of infections of nosocomial origin. It is considered as one of the most challenging pathogens globally because of its high rate of resistance to antimicrobial agents. The injudicious use of antibiotics in our country has resulted in increased resistance to commonly prescribed antibiotics which have consequently greater mortality and morbidity as well as high cost to treat infectious diseases. The gender distributions of our study showed that 57% of isolates were from males while the other 43% from females. This finding is consistent with studies around the globe which reported the higher isolation rate from males and it is supported by study from Iraq and a study from Karachi [8,14].

According to our study, among 289 positive isolates, 58 were identified as *P. aeruginosa*. The frequency of *P. aeruginosa* in pus specimens is calculated to be 20% which is in agreement with study from India by Verma [4] who reported the frequency of *P. aeruginosa* in pus specimens to be 18%. The similar frequency i.e. 19% was obtained by Ojentibeju and Nwobu [15] from Nigeria. Another study from Nigeria revealed that the frequency of *P. aeruginosa* in pus specimens was 11% which is not in agreement with our study Idowu et al., [16]. Considerably different results were obtained by Ranjan, et al. [17] from India, who concluded that *P.aeruginosa* has the frequency of 30% in pus specimens. Jamsahaid, et al. [14] from Pakistan determined the frequency around 6.67% which is very less as compared to our results. From America, Masaadeh, et al. [18] found that the frequency of *P. aeruginosa* was 28%. Aminizadeh and Sadat [19] from Iran reported the prevalence of *P. aeruginosa* as 13.2%.

According to results of our study, gentamicin was found to be the least effective antibiotic against *P. aeruginosa*. Among 58 isolates tested, half of *P. aeruginosa* isolates (50%) were susceptible while other half (50%) were resistant to gentamicin which indicates the development of resistance to it. A study from Karachi reported the susceptibility of 59% isolates of *P. aeruginosa* to gentamicin [20] while from India a researcher reported the susceptibility of 60% isolates to gentamicin [21]. However, a very high rate of susceptibility was shown by gentamicin as reported from Malaysia (85% isolates susceptible) and United Kingdom (87% isolates susceptible) which is quite a higher susceptibility rate as compared to our results [22,23].

In this study, we found that 64% isolates tested were susceptible while 36% isolates showed resistance to amikacin. Our results are in concordance with results of study from Karachi which reported the same susceptibility rate (64% isolates susceptible) of amikacin to *P. aeruginosa* isolates [20]. Similar study from Egypt revealed that resistance to amikacin was 44% [24]. In contrast, a very high susceptibility rate of 94% with 652 isolates susceptible out of 692, was reported from Japan and low susceptibility rates of 18% was reported from Iraq [8,25]. In our study, 34% isolates were resistant to ciprofloxacin. This resistance rate is in accordance with studies from India and Nepal which reported the resistance rate to be 39% and 28% respectively [21,26]. A researcher from Karachi also reported the exact same resistance rate i.e. 34% [20]. The resistance rate is 8% in Turkey, 11% in the United Kingdom and 16% in Malaysia which is in contradiction with results of our study [22,23].

Our study revealed that the resistance rate to aztreonam was 34% which is perfectly in agreement with the resistance rate reported from Japan (32%) and India (39%) [27]. The resistance rate from Iran is reported to be 20% which is less than the resistance rate claimed by our study [28]. According to our study, ceftazidime showed great susceptibility rate against *P. aeruginosa* isolates. Only 15% isolates were resistant to it. The low resistance rate is comparable to the resistance rate reported from the United Kingdom (11%), Turkey (13%) and Japan (16%) [23,25]. Significantly higher resistance rates are reported from Iran (69%) and India (70%) which are contradictory to our results [29,30]. It also highlights the fact that emergence of resistance to third generation cephalosporins like ceftazidime needs immediate attention.

Results of our study revealed that 31% isolates were resistant to cefoperazone. This finding is supported by a study from India which reported the resistance rate of *P. aeruginosa* isolates to cefoperazone to be 33% [31]. Another study from India contradicts our results and it has reported the resistance rate to be 69% which is way too much as compared to the resistance rate provided by our study [29]. The lower resistance rate (15%) has been reported from the United Kingdom [23]. Tazocin is a combination of penicillin 'piperacillin' and beta-lactamase inhibitor 'tazobactam'. In this study, 29% isolates were found to be resistant to tazocin. The researchers from Japan and India have also reported the similar resistance rates of 29% and 34% respectively [25]. According to a study conducted in India, the resistance rate was reported to be 4% which is not in accordance with results of our study [29].

According to our study, colistin was the most effective antipseudomonal antibiotic as 88% isolates were susceptible to it. Only 12% isolates exhibited resistance to it. Recently a study from India reported the resistance rate to be 13% which is in concordance with results of our study [21]. Researchers from the United Kingdom reported that among 505 clinical isolates of *P. aeruginosa*, all were susceptible to colistin and none was found resistant to it [23]. Results of our study revealed that meropenam exhibited significant antipseudomonal activity as 79% isolates were susceptible with only 21% isolates exhibiting resistance to it. This finding has been supported by results of other studies from different parts of the world which also reported similar resistance rate. The resistance rate is 16% in India, 21% in Japan and 23% in Malaysia [22,25,32]. In a study from Karachi, the resistance rate of *P. aeruginosa* isolates from patients with chronic suppurative otitis media was 24% [33]. A study from Egypt has reported a very high resistance rate of 68% to meropenam by *P. aeruginosa* isolates which contradicts the with the result of our study [34].

Sulzone is a combination of cephalosporin 'cefoperazone' and beta-lactamase inhibitor 'sulbactam'. According to our study, the resistance rate to sulzone was found to be 21% which indicates a significant activity against the *P. aeruginosa* isolates. Studies from Japan and India validate our results as they have reported the resistance rate to be 22% [21,27]. The resistance rate in Nepal has been reported to be 34% and in the United Kingdom 40% which is higher than the resistance rate revealed by this study [23]. According to our study, among 58 isolates of *P. aeruginosa*, 19 were classified as multidrug resistant i.e. resistant to at least 3 different classes of antimicrobial agents. The prevalence of MDR *P. aeruginosa* was found out to be 32.7%. Researchers from Iran revealed the prevalence of MDR *P. aeruginosa* to be 28% which is slightly less than prevalence found by our study [28]. A study from Malaysia reported the prevalence of MDR *P. aeruginosa* to be 19.6% as 19 isolates out of 97 were multidrug resistant [22]. Another study from India has reported a higher prevalence (45.2%) of MDR *P. aeruginosa*. Out of 53 isolates, 24 were found out to be multidrug resistant [35]. The prevalence of MDR *P. aeruginosa* has been reported to be 42.3% in burn patients which is not in accordance with our results [30].

Conclusion

We can conclude from our study that the frequency of *P. aeruginosa* isolated from pus specimens is 20%. Antimicrobial susceptibility pattern of *P. aeruginosa* isolates showed that colistin is the most effective antimicrobial agent against *P. aeruginosa* followed by ceftazidime, meropenam and sulzone. None of the antimicrobial drugs exhibited 100% susceptibility rate. Gentamicin is found to be least effective. Moderate antipseudomonal activity is shown by other antimicrobial agents such as ciprofloxacin, aztreonam and amikacin. Low resistance rate is obtained against cefoperazone and tazocin. Study also revealed the prevalence of MDR *P. aeruginosa*. 32.7% isolates are classified as MDR strains.

Findings of study need to be interpreted in the light of certain limitations as under.

- Results are limited to the individuals assessing to the Pathology department of Holy Family Hospital Rawalpindi. So, results of study cannot be generalized to the overall population.
- Modified Kirby Bauer disk diffusion method was used for Antimicrobial susceptibility testing. Further studies may be carried out using MIC by either broth or agar dilution which is standard procedure.
- Study was conducted by using a small sample size. Studies with large sample size are required to accurately assess the susceptibility profile of isolates.

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