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Pseudomonas aeruginosa Infection and Antibiotic Sensitivity Pattern Isolated from a Tertiary Care Hospital in Dhaka City

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Pseudomonas aeruginosa is one of the most widespread gram-negative microorganisms identified in the clinical samples and most common causes of hospital acquired infection. *P. aeruginosa* is affecting both indoor and outdoor patients throughout the world. Due to frequent mutation in *P. aeruginosa* highly resistant strain developed rapidly. The aim of the study to determine the prevalence of *P. aeruginosa* species in different samples isolated from a Tertiary care Hospital as well as determination their diverse antibiotic resistance pattern. This cross-sectional study was carried out to determine in-vitro resistance pattern of *P. aeruginosa* isolates to common antimicrobial agents by disc diffusion method. Various clinical samples were collected from Bangladesh Health Sciences Hospital (BIHS) General Hospital, Dhaka. This research was carried out in the Department of Microbiology of Bangladesh University of Health Sciences (BUHS). Isolation, identification and antibiogram were performed for *P. aeruginosa* following standard microbiological laboratory procedure. A total of 218 *P. aeruginosa* were isolated from 3062 different

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clinical specimens which are statistically significant (p<0.0001). Among the highest number of *P. aeruginosa* were isolated from outdoor patients 140 compare to Indoor patients which are significantly higher (p <0.013). In this study Male (68.3%) are more vulnerable to *P. aeruginosa* infection compare to females (31.7%) which is also statistically significant. Young people (less than 35 years) were more susceptible to *P. aeruginosa* infection which is also statistically significant (p< 0.01). The highest number of *P. aeruginosa* was isolated from wound (43.12%), followed by pus (40.33%), sputum (8.71%) urine (7.80%). The maximum number of *P. aeruginosa in various samples* was resistant to aztreonam and co-tromoxazole followed by cephalosporins, aminoglycosides, carbapenems. The most sensitive antibiotic was colistin of followed by gentamycin and tetracycline. To control the spread of resistant bacteria, it is disparagingly vital to have stringent antibiotic guidelines. The antibiotic susceptibility pattern of *P. aeruginosa* requires to be continuously monitored in specialized clinical units and the results readily made available to the clinicians to minimize the resistance.

Keywords: Pseudomonas aeruginosa' AmpC beta-lactamase; muller-hinton agar media; antimicrobial susceptibility testing; colistin.

1. INTRODUCTION

P. aeruginosa is found in the hospital environment and therefore it is the most common causative agent of nosocomial infections. The bacterium possesses a wide range of secretion systems, which secretes numerous proteins relevant to the pathogenesis of clinical strains [1]. *P. aeruginosa* is found throughout the body, most commonly in the urinary tract, respiratory tract, blood, and wounds [2]. *P. aeruginosa* is a type of bacterium that can develop resistance to antibiotics rather rapidly over several generations. This resistance present in some strains makes *P. aeruginosa* very difficult to treat infected hosts such as a human or other animal [3]. Production of a variety of beta-lactamases, outer membrane permeability and combinations of multiple mechanisms of resistance, the gram-negative bacteria are becoming resistant to beta lactam drugs such as cephalosporins, monobactams and carbapenems [4]. Some strains of *P. aeruginosa*produce AmpC beta-lactamase enzyme and this enzyme make this strain MDR (multi drug resistance) bacterium. As *P. aeruginosa* is a commensal in healthy people the frequency of commensalism risesgradually with the duration of hospital stay [5]. *P. aeruginosa* is frequently isolated as an opportunistic pathogen in occurring often infection of hospitalized patients and has been isolated from a number of sites in the hospital environment [6]. *P.aeruginosa* is the most important, resistant and dangerous organism infecting the burn patient [7]. It is the fifth common pathogen among hospital microorganisms and causes 10% of all hospital acquired infection [8]. In Bangladesh, it ranks third and causes a wide range of infection [9]. Recently this bacterium has become increasingly resistant to various antimicrobial agent [10]. Drugs resistant *P.aeruginosa* isolated has emerged and continues to escalate rapidly [11]. Over the period of time we observed an increase in the number of *P. aeruginosa* among laboratory isolates so we decide to carry out a cross-sectional study to see infections caused by *P. aeruginosa* and the susceptibility pattern of the organism isolated from a different clinical specimens at the department of microbiology, Bangladesh University of Health Science.

2. MATERIALS AND METHODS

This cross-sectional laboratory-based retrospective study was carried out in the Department of Microbiology, BUHS from 1st July 2017to 31st August 2018. A total of 218 *P.aeruginosa* were isolated from 3062 clinical specimens including wound swab, pus, urine, sputum. To avoid contamination, wounds and pus samples were thoroughly cleaned with sterile normal saline followed by gentle rubbing of the wound site with 70% alcohol before swabbing samples. Urine and sputum samples were also collected by maintaining an appropriate procedure. The isolates were identified by colony characters, Gram's staining and different biochemical test were performed; Triple sugar iron, citrate, Motility, Indole, Urea and the Confirmatory test for *P.aeruginosa* was performed Oxidase positive test (In case of blood, pus wound swab and urine sample). The antibiotic susceptibility patterns of *P.aeruginosa* isolates were analyzed by clinically used common antibiotics. Antibiotic Susceptibility test was carried out by disc diffusion method (Kirby-Bauer) in Muller-Hinton agar media according to

the CLSI. M100 Performance Standards for Antimicrobial Susceptibility Testing, 30th edition. The results of the susceptibility test were interpreted into Sensitive, Intermediate and resistant [12].

3. RESULTS

The cross-sectional study was conducted on 3062 patients. Among 3062 samples, 218 *P.aeruginosa* were isolated and the proportion was 7.1 (95% CI 6.2 to 8.0) (p<0.0001). Where most of the *P.aeruginosa* (64.2%) was isolated from outdoor patients and 35.8% were from indoor patients, which is significant. Maximum (86.2%) patients were estimated above 35 years, and the rest of (13.8%) were below 35 years. 68.3% *P. aeruginosa* were predominantly isolated from male patients and female patients 31.7% (Table 1).

Among 218 isolates of *P. aeruginosa,* 94 were isolated wound samples (43.12%) which is highest in number following, 88(40.33%) from pus, 19 (8.71%) from sputum and 17(7.80%) from urine (Table 2).

The resistance rate of *P.aeruginosa* isolated from different specimens to different antimicrobials wide-ranging from 0.01% to 100%. *P. aeruginosa* isolated from pus, wound and urine sample were found 100% resistant to aztreonam and co-trimoxazole. Isolates of pus samples found (84.1%), (89.7%), (82.9%) resistance to ciprofloxacin, doxycycline and cefixime antibiotics respectively. *P. aeruginosa*of pus and wound samples were found (84.1%) and (64.8%) resistance to carbapenem groups of antibiotics respectively. However, pus isolates were found (94.3%) sensitive to Colistin. *P. aeruginosa* isolated from wound samples were estimated (95.7%) and (92.6%) resistance to ceftazidime and doxycycline respectively. *P. aeruginosa* isolated from sputum samples were assessed (89.5%) susceptible to imipenam, gentamicin, amikacin and tigecycline. However, *P. aeruginosa* isolated from sputum samples were found (100%) resistance to aztreonam. *P. aeruginosa* isolated from urine samples were found (88.2 %) sensitive to gentamicin and amikacin and (88.2%), (94.1%) and (100%) resistance to ceftazidime, doxycycline, co-trimoxazole and aztreonam respectively. On the other hand, *P. aeruginosa* isolated from urine and sputum samples were found (82.4%) and (89.5 %) sensitive to carbapenem groups of antibiotics respectively. Where colistin were found more effective drug against all kind of *P. aeruginosa* infection. *P. aeruginosa* isolates of wound sample were found (71.3 %) sensitive to colistin. *P. aeruginosa* isolated from sputum samples were found to (99%) sensitive to colistin (Table 3).

4. DISCUSSION

P. aeruginosa emerged as a common pathogen for nosocomial infections. It is one of the important causes of illness among indoor and outdoor patients in the hospital. The increasing resistance rate of *P. aeruginosa* in hospital infections is due to its incomplete and indiscriminate use of antibiotics. In the present study, it is manifest that there is diversity in the resistant pattern of isolates of *P. aeruginosa* from different clinical samples. In our study, the maximum of *P. aeruginosa* were identified from patients who were aged above 35 years which is 188 (86.2%) compared to below 35 years which is 30 (13.8%). However, the study of The National Nosocomial Infections Surveillance System showed that aged between 21-40 years (41.2%) were more susceptible to *P. aeruginosa* infection [13]. Where male patients were more vulnerable to *P. aeruginosa* infection 149 (68.3%) comparison to female 69 (31.7%). In 2004 Friedland et al. showed a prevalence of *P.aeruginosa* species in outdoor patients was 70% and indoor patients were 30% [14]. In our study, we found the same parameter where outdoor patients (64.2%) prevalence is higher in reference with indoor patients (35.8%) with an increasing rate.

Table 1 Distribution of patients with *P. aeruginosa* infection

Variables		Number	Percentage	P value
Patient group	Indoor patient	78	35.8%	< 0.013
	Outdoor patient	140	64.2%	
Gender	Male	149	68.3%	< 0.01
	Female	69	31.7%	
Age group	Below 35	188	86.2%	< 0.01
	Above 35	30	13.8%	

Table 2. Percentages of *P. aeruginosa* isolated from both indoor and outdoor patients different clinical samples (n=218)

Type of sample	No. of sample (n)	No of *P.aeruginosa* isolated	Percentages (%)
Urine	2116	17	7.80%
Pus	455	88	40.33%
Wound	306	94	43.12%
Sputum	185	19	8.71%
Total	3062	218	100%

Table 3. Resistant pattern of *P. aeruginosa* to commonly used antibiotics (n=218)

Antimicrobials	Resistance Proportion of Wound *P. aeruginosa* (n=94)	Resistance Proportion of Urine *P. aeruginosa* (n=17)	Resistance Proportion of Pus *P. aeruginosa*(n=88)	Resistance Proportion of Sputum *P. aeruginosa*(n=19)
Imepenam	64.8%	17.6%	84.1%	10.5%
Ciprofloxacin	81.0%	35.3%	84.1%	15.8%
Ceftazidime	95.7%	88.2%	86.3%	68.4%
Doxycycline	92.6%	94.1%	89.7%	73.7%
Gentamicin	79.8%	11.8%	76.1%	10.5%
Cefixime	89.4%	94.1%	82.9%	63.2%
Amikacin	76.6%	11.8%	73.9%	10.5%
Co-trimoxazole	100%	100%	100%	63.2%
Aztreonam	100%	100%	100%	100%
Tetracycline	36.2%	23.5%	23.9%	10.5%
Colistin	28.7%	5.9%	5.7%	0.01%

Maximum clinical isolates of *P.aeruginosa* were isolated from the wound (43.12%), followed by pus (40.33%), urine 17 (7.80%). These results are in line with studies of Houqe MM et al. where they estimated (pus/ wound 38.38% and urine 28.28%) [15]. Similarly, Mousumi et al. in found the prevalence of *Pseudomonas* species in diabetic foot infected patients where pus samples were 22% and in case of non-diabetic foot infected patients where pus samples were 29% [16]. Though, in the study results of Teteeng et al. contradict our results they estimated, 19% *P. aeruginosa* from wound samples, and they identified the highest number of *staphylococcus aureus* (36%) from wound samples, these data contrast to our study [17]. In this study in case of sputum sample, only 8.71 % *P. aeruginosa* were isolated, similarly, Abdelraouf et al. identified 7.0% *P. aeruginosa* in sputum samples [18]. In this study, *P.aeruginosa* isolated from wound samples and pus were found more resistant in all commercially available antibiotic compare to *P. aeruginosa* isolated from urine and sputum samples. Most of the *P. aeruginosa* species isolated from all samples were found resistance to co-trimoxazole and aztreonam. On the other hand, Abdelraouf et al. found *P.aeru*ginosa highly resistant to amoxicillin and cephalexin [18]. In our study, *P. aeruginosa* isolated from pus samples were found 100% resistance to co-trimoxazole and aztreonam, 86.3% doxycycline, 82.9% cefixime, and 84.1% resistance to both imipenem and ciprofloxacin, 76.1% gentamicin, 73.9% amikacin. In 2015 Mousumi et al. estimated that only 33% *P. aeruginosa* were resistant to carbapenem groups of antibiotic which is now 100% [16]. Which indicates carbapenem resistant is increasing day by day. Though only 5.7% *P. aeruginosa* pus samples were found resistance to colistin. Teteeng et al. found only 8% ciprofloxacin resistance *P. aeruginosa* from wound samples. But comparable to our study Siddiqua et al. showed highest resistance (91% to 96%) to cotrimoxazole and cefuroxime *P. aeruginosa* isolated from wound samples [19]. *P. aeruginosa* isolated from urine samples were found 100% resistance to azitronam and co-trimoxazole as well as 94.1% to doxycycline and cefixime. 17.6% resistance to imipenem. Alike our study Ali H et al. found almost all of the *P. aeruginosa* isolates from urine samples were highly resistant

to doxycycline but sensitive to amikacin (100%), norfloxacin (86%) ciprofloxacin (83%) and tobramycin (83%) [20]. Though, Fatima et al. estimated the highest resistance to *P. aeruginosa* isolate with piperacillin/ tazobactam and cefipime i.e. 42% and 40% respectively. Imipenem was found to be most effective antibiotic against *P aeruginosa* (76% sensitivity) but amikacin resistance was continuously increasing [21]. In our study, we also found imipenem and colistin also found effective drug against *P aeruginosa* isolated from sputum samples. Colistin (99.6% sensitive) proved to be one of the most effective drugs for routine use among the *P. aeruginosa* strains investigated in this study. Colistin therapy was at least as effective and as safe as beta-lactam antibiotic or a quinolone in the treatment of infections caused by multidrug-resistant *P. aeruginosa* [22].

5. CONCLUSION

In summary, *P. aeruginosa* strains were more commonly isolated from pus and wound samples. Carbapenem resistance *P. aeruginosa* strains are increasing day by day and our study also demonstrated that colistin is highly useful as a preferred and effective drug for all kinds of *P. aeruginosa* infection. Though resistance to colistin of pus sample is lower in comparison with other antibiotics, but it is really an issue of concern to found resistance to colistin antibiotic. The superiority of colistin as anti-pseudomonal therapy needs to be further verified through larger prospective clinical trials. To prevent the spread of resistant bacteria, it is critically important to have strict antibiotic guidelines while investigation programs for multidrug-resistant organisms and infection control procedures need to be executed. It is compulsory that the antibiotic sensitivity pattern of highly resistant bacterial pathogens like *P. aeruginosa* in particular clinical units to be continuously supervised and the results readily made accessible to the physician to control the morbidity.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

We take consent from every patient during data and sample collection.

ETHICAL APPROVAL

This research was approved by the Ethical committee of Bangladesh University of Health Sciences.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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