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Original Article

Pseudomonas Aeruginosa Continues its March on the Road of Antibiotic Resistance in Pakistan

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ABSTRACT

Objective: The main aim of study wasto find the frequency and to determine the current susceptibility pattern of multi-drug resistant (MDR), extensively drug resistant (XDR) and pan drug resistant (PDR of *Pseudomonas aeruginosa* isolated from different clinical specimens.

Material and Methods: It was a cross-sectional single center study. This study was carried out at Microbiology department of Army Medical College (National University of Medical Sciences)/Pak Emirates Military Hospital (PEMH) Rawalpindi, Pakistan from March 2019 to Aug 2019 after obtaining permission from ethical review board of institute.

All clinical samples received in microbiology laboratory for culture and sensitivity of *Pseudomonas aeruginosa* were included except for urinary catheter and endotracheal tips. Exclusion criteria consisted of duplicate samples of same patient. Specimens were inoculated on different culture media like Blood agar, MacConkey agar depending on requirement of specimen. Gram stain, colony morphology was done initially and standard microbiological methods like oxidase test, catalase test and Analytical profile index (api-20 NE) biomerieux was used for identification of isolates. Isolates were identified till species level. Kirby-Bauer modified disc diffusion method was used to find the antibiotic susceptibility and results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: During the study period, 157 *Pseudomonas aeruginosa* were detected from different clinical specimens. Multi drug resistant *Pseudomonas aeruginosa* was detected in 37 (23.6%) isolates. Frequency of extensively drug resistant *Pseudomonas aeruginosa* was found to be 20 (12.7 %). Pan drug resistance was seen in 4 (2.5%) percent isolates of *Pseudomonas aeruginosa.* Colistin was found to be most sensitive drug i.e. (75.6%) in multi drug resistant and (90%) in extensively drug resistant isolates of *Pseudomonas aeruginosa.* After Colistin in multidrug resistant strains Piperacillin-Tazobactam (75.6%) and Meropenem (62.1%) were sensitive whereas extensively drug resistant strains were most susceptible to Colistin (90%) and Aztreonam (15%).

Conclusion: Multi drug resitant infections are on rise. Pseudomonas *aeruginosa* infections that are multi drug resistant and extensively drug resistant are occurring at a significant rate and are leading towards era of ineffective and limited therapeutic options which can be avoided by adopting antibiotic stewardship and creating more awareness about antibiotic resistance and adhering to local antibiogram.

Key words: Antimicrobial resistance, extensively drug resistant Pseudomonas aeruginosa, Multidrug resistant *Pseudomonas aeruginosa*

Introduction

Pseudomonas aeruginosa causes a wide range of infections in immunocompromised individuals It is an opportunistic and hardy pathogen which causes infection mostly in nosocomial settings.

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Appearance of highly resistant strains of *Pseudomonas aeruginosa* is a significant health challenge that needs to be managed on priority as treatment options are fast depleting posing a threat to mortality and morbidity. Scarce treatment options for these infections is a global threat as according to the World Health Organization 2017 data carbapenem-resistant *Pseudomonas aeruginosa* was included to the "critical" group list for which new antibiotics are immediately needed.[1]

It is Gram-negative, aerobic non fermenting bacillus, commonly causing infections in hospitals but especially in intensive care units where it causes life

threatening infections such as blood stream infections, septicemia, ventilator associated pneumonia, endocarditis, surgical site infections and urinary tract infections.[2]

Pseudomonas aeruginosa possess extraordinary ability to resist a wide range of antimicrobials hence making its complete extinction laborious. Resistance in *Pseudomonas aeruginosa* occurs through various mechanisms i.e. intrinsic resistance due to efflux pump over expression and less permeable outer membrane. Horizontal transfer of gene, mutations in genes encoding porins, efflux pumps and penicillin-binding proteins confer acquired antibiotic resistance to *Pseudomonas aeruginosa*.[3] Lastly resistance also occurs through adaptive mechanism i.e. formation of biofilm. All the above mechanisms act synergistically leading to emergence of MDR, XDR AND PDR *Pseudomonas aeruginosa* strains. [4, 5]

Therapeutic options for resistant *Pseudomonas aeruginosa* according to CLSI are mostly limited to β-lactams (i.e. carbapenems, β-lactam inhibitors and third generation cephalosporins like ceftazidime), which are used in combination with other agents such as group B (aminoglycoside, fluoroquinolone), and group O (polymyxin) drugs.[6] In addition to conventional agents several novel combinations like ceftolozane-tazobactam, ceftazidime-avibactam or newer drugs like cefiderocol can also be used.[7]

Development of new effective antimicrobial agents and to preserve already available treatment options is need of hour but that process occurs at slow pace where as these bacteria continue to acquire resistance at an intensely rapid pace. The only solution remains the knowledge of local antibiotic susceptibility pattern of different highly drug resistant strains of *Pseudomonas aeruginosa* isolates to apply antibiotic stewardship and the judious use of effective antibiotics.

Material and Methods

Pak Emirates Military Hospital (PEMH) is an eleven hundred bedded tertiary care hospital, with a heavy patient turn over so we were able to isolate 157 *Pseudomonas aeruginosa* in six month time, from clinical samples submitted to the laboratory of department of microbiology. This cross-sectional single center study spanning over six months was carried out at department of microbiology Army medical college /PEMH (National university of Medical Sciences) from march 2019 to august 2019. The study was done after obtaining ethical approval from institutional review board of Army Medical College IERB no 118

We included 157 isolates of *Pseudomonas aeruginosa* in our study as calculated by using WHO calculator for sample size calculation by keeping confidence interval at 95 %.The sampling technique used was Non-probability convenience sampling technique. Samples comprised of pus, urine, sputum, bronchoalvelolar lavage, blood, body fluids and swabs from vagina and ear. Endotracheal tips, urinary catheter tips and duplicate samples from the same patient were excluded.

Colony morphology, pigment production, gram staining, oxidase test, catalase tests and other routine preliminary microbiological tests were used for bacterial identification which was confirmed by using analytical profile index (api-20 NE) biomerieux.

Kirby-Bauer modified disc diffusion method using the Cationic Mueller Hinton agar was performed using the Clinical and Laboratory Standards Institute (CLSI) guidelines to determine the Antibiotic susceptibility of isolates. McFarland 0.5 standard was used. Antibiotic discs of Piperacillin/ tazobactam (TZP 100/10ugm), Meropenem (MEM 10µgm), Aztreonam (AZT 30 µgm), Ceftazidime CAZ 30µgm), Amikacin (AK 30µgm), Gentamicin (CN 10µgm), Ciprofloxacin (CIP 5µgm), Levofloxacin (LEV 5µgm) were placed on agar plate. Plates were incubated at 35 C for 18±2 h. Zones of growth inhibition around each of the antibiotic disc were measured and named as either sensitive or resistant according to CLSI guidelines.Colistin agar was used to determine resistance of Colistin.

For the purpose of this study we defined MDR *Pseudomonas aeruginosa* as MDR as a strains that are resistant to more than three or more antimicrobial drug classes. XDR *Pseudomonas aeruginosa* was defined as isolate which is resistant to all drug classes except two. Isolate resistant to all drug classes is referred to as Pan-drug resistance[8, 9].

Data was analyzed by using Statistical Package for Social Sciences (SPSS) version 26. Frequency and percentages were calculated for categorical variables.

Results

During study period different clinical samples yielded 157 isolates of *Pseudomonas aeruginosa.* Specimens ranged from pus, respiratory, blood, ear swab, high vaginal swab to urine and fluids as shown in figure

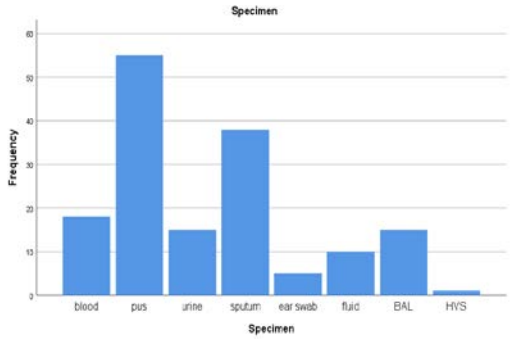

Fig 1: Frequency of specimens from different sites.

The age of our patients ranged from 20 years to 60 years with most isolates obtained from outdoor department followed by medical wards and medical intensive care unit. From outdoor department 77 (49%) samples yielded *Pseudomonas aeruginosa* followed by medical ward which yielded 37 (23.6%),than medical intensive care unit 20 (12.7%), Liver transplant unit 10 (6.4%) neonatal intensive care unit 12 (7.6%) followed by 1(0.6%) from surgical ward as shown in table 1

Table 1: Distribution of *Pseudomonas aeruginosa* isolated from Clinical specimens (n=157) from different units of hospital

Hospital units	Clinical specimen (%)
Outdoor department	77 (49)
Medical ward	37 (23.6)
Medical intensive care unit	20 (12.7)
Liver transplant unit	10 (6.4)
Neonatal intensive care unit	12 (7.6)
Surgical ward	1 (0.6)

Out of 157 isolates of *Pseudomonas aeruginosa* the percentage of MDR *Pseudomonas aeruginosa* was 37 (23.6%), XDR 20 (12.7%) and PDR 4 (2.5%) were respectively. Distribution of *Pseudomonas aeruginosa* according to specimens as shown in table 2

Table 2: MDR, XDR and PDR *Pseudomonas aeruginosa* distribution according to clinical specimens

Specimen	MDR 37 (23.6%)	XDR 20 (12.7%)	PDR 4 (2.5%)
Pus	13	4	1
Blood	4	4	-
Urine	4	2	3
Sputum	4	2	-
Ear swab	2	-	-
Fluid	5	-	-
Bronchoalveolar lavage	5	8	-

The antibiogram showed that Colistin and piperacillin-tazobactam has highest susceptibility followed by meropenem and amikacin in MDR strains of *Pseudomonas aeruginosa* isolates as shown in table 3.

Table 3: Antibiogram of MDR *Pseudomonas aeruginosa.*

Anti-pseudomonal antimicrobial agents	Antibiotic susceptibility of MDR Pseudomonas aeruginosa
Colistin	75.6%
Piperacillin -Tazobactam	75.6%
Meropenem	62.1%
Amikacin	48.6%
Ciprofloxaxcin	43.2%
Gentamicin	35.1%
Ceftazidime	27.0%
Aztreonam	13.5%

Table 4: MDR, XDR and PDR *Pseudomonas aeruginosa* exhibiting resistance against different anti-pseudomonal antibiotics

Antibiotics	Percentage resistance among MDR n=37	Percentage resistance among XDR n=20	Percentage resistance among PDR n=4
Colistin	13.5%	0	100%
Piperacillin - Tazobactum	24.3%	85%	100%
Meropenem	37.8%	95%	100%
Amikacin	51.3%	95%	100%
Ciprofloxacin	54.0%	95%	100%
Gentamicin	64.8%	100%	100%
Ceftazidime	72.9%	95%	100%
Aztreonam	86.4%	85%	100%

Table 4 shows the percentage of MDR, XDR and PDR *Pseudomonas aeruginosa* against antipseudomonal antimicrobials where the highest resistance is exhibited by aztreonam, ceftazidime and gentamicin. Colistin (75.6%), piperacillin-tazobactam (75.6%) followed by meropenem (62.1%) and amikacin (48.6%) were found to be most sensitive to MDR strains of *Pseudomonas aeruginosa.* Colistin and aztreonam are still the only effective agents against XDR *Pseudomonas aeruginosa.* Colistin is effective against 90% and aztreonam against 15% *Pseudomonas aeruginosa* XDR isolates.

Discussion

Antimicrobials against *Pseudomonas aeruginosa* infections are becoming increasingly ineffective due to growing multidrug resistant strains. This rising curve in antibiotic resistance pattern is multifactorial. It occurs through innate, acquired or adaptive mechanisms. Presence of variety of antibiotic resistant mechanisms especially formation of biofilm and persister cell, lack of development of new antibiotics, lack of implementation of antibiotic stewardship and versatile nature of *Pseudomonas aeruginosa* all have led to origin of multidrug-resistant strains.[5]

In our study the frequency of MDR and XDR *Pseudomonas aeruginosa* was 23.6% and 12.7% respectively. A similar study conducted in Rawalpindi in past showed the frequency of MDR *Pseudomonas aeruginosa* to be around 22% and XDR *Pseudomonas aeruginosa* to be *11 %*. [10] A research group from Islamabad, Pakistan showed the presence of MDR and XDR *Pseudomonas aeruginosa* to be 36.3% and 18% respectively[11] The frequency of MDR *Pseudomonas aeruginosa* was 36.89% according to a study conducted by ijaz et al. [12] A similar study conducted in Peshawar, Pakistan showed the frequency of MDR *Pseudomonas aeruginosa* to be 25% and XDR to be 10.7%[13].Additionally studies from south East Asia exhibit comparable results. A study from India showed 35.4% isolates to be MDR and 23.6% isolates were XDR.[14] Another study conducted in India showed the prevalence of XDR *Pseudomonas aeruginosa* to be around 12.1%.[15] Resistance to *Pseudomonas aeruginosa* is increasing at an alarming rate. Global statistics are also similar.[1,16] This increase is worrisome especially in developing countries where there is lack of resources, unchecked availability of antibiotics, lack of education and awareness all leading to development of resistant strains.

Our study showed MDR strains had highest susceptibility to colistin, piperacillin-tazobactam, followed by meropenem and amikacin. Aztreonam exhibited least susceptibility followed by ceftazidine, gentamicin and ciprofloxacin respectively indicating limited treatment options. A similar study conducted in our setup in 2011 in which strains showed susceptibility to colistin, piperacillin-tazobactam followed by cefoperazone-sulbactam ,Ceftazidime and cefoperazone respectively.[10] *Pseudomonas aeruginosa* has developed almost complete resistance against cefoperazone-sulbactam, ceftazidime and cefoperazone. Resistance against meropenem has fallen from 85.5% resistance in 2011, to 37% now most probably of its restricted use in chemical practice[19]

XDR *Pseudomonas aeruginosa* strains show very high resistance to almost all classes of antibiotics with colistin being last resort. High resistance found in our study is suggestive of emergence of MDR and XDR pathogens.[17]

A wide variety of *Pseudomonas aeruginosa* isolates were obtained from pus followed by body fluids. Similar pattern was seen in earlier studies conducted in Pakistan. [18,19] Studies conducted in India followed same pattern having highest percentage of *Pseudomonas aeruginosa* in pus specimens followed by body fluids [15] where as Gallagher et al. showed respiratory tract to be most common site of infection [20] .An unrestrained, freely available over the counter antibiotics, over prescription of antibiotics, free use of broad spectrum antibiotic and widely occurring infectious diseases in developing world all contribute to the increased drug resistance and emergence of multidrug resistant, extensively drug resistant and total drug resistant organisms[21]. Therefore there is need of antibiotic stewardship in our part of world.

Conclusion

Over the past few years resistance in *Pseudomonas aeruginosa* has increased unchecked leading to emergence of MDR, XDR and PDR strains. Treatment options are fast depleting and colistin is becoming the last resort for treatment of MDR and *XDR Pseudomonas aeruginosa.* Hence it is recommended that use of broad spectrum antibiotics should be carried out with restraint reserving antibiotics use for severe life threatening infection.Regular laboratory detection, surveillance education and awareness regarding antibiotic stewardship is need of hour to combat this pathogen.

Limitations

The main limitation of study was we did not determine the mean inhibitory concentration of Colistin. Moreover a study should be done on larger scale at multicenter level to combat rising resistance in *Pseudomonas aeruginosa*.

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