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STUDY ON ANTIBIOTIC PROFILE OF MDR PSEUDOMONAS AERUGINOSA (MDRPA) ISOLATES FROM BURN PATIENTS: TIP OFF TREATMENT OF CHOICE.

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ABSTRACT Burn wards harbour MDR pseudomonas aeruginosa which subsequently colonize burn wounds leading to life threatening infection. MDRPA strains pose a challenge in selecting the most appropriate antibiotics worldwide. This study was undertaken to identify the isolation rate of MDR Pseudomonas aeruginosa and its antibiotic profile among the burns patients. Materials and Methods: This study was conducted over a period of 6 months in a burn unit of Government medical college Hospital and Research Institute, Chennai. 267 consecutive clinical samples from burn unit were collected during this period. The isolates were identified and antibiotic sensitivity testing of all P.aeruginosa isolates was done using Kirby-Bauer disc diffusion method and the results were interpreted according to the CLSI guidelines. Ceftazidime resistant strains were subjected to MIC by agar dilution method. Results: Out of 267 clinical samples, 108 P. aeruginosa was isolated. Among them, 35.18% was found to be MDRPA. 55% were resistant to beta-lactams - piperacillin, 26% were resistant to piperacillin-tazobactam, 19% were resistant to ciprofloxacin and 2.36% were resistant to carbapenems. All isolates were resistant to ceftazidime (100%).MIC of Ceftazidime revealed majority of the strains had breakpoints at 64µg/ml. Conclusion: Periodic review of antibiotic profile of MDRPA in burn patients should be the leading priorities to curtail the menace of resistance.

KEYWORDS : MDRPA, Antibiotic profile, MIC ceftazidime, Burn patients

INTRODUCTION

Pseudomonas aeruginosa well recognized as an epitome of opportunistic infections, known for posing significant challenge in burn wound infections. According to infectious disease society of America, ESKAPE *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species)* group of pathogens are commonly implicated in nosocomial infections. P. aeruginosa, familiar to every hospitalist and intensivist accounts for 10-20% nosocomial infections in the form of septicaemia in intensive-care units, cystic fibrosis, burn and wound infections[1].

Extensive breaches in the skin barrier, immune suppression in burn patients in addition to the fact that *Pseudomonas aeruginosa* occurs so commonly in the environment with predilection for dead, denatured tissues poses a great challenge for burns patients.

The scenario is further compounded by its inherent resistant as well as acquired resistance to many effective antimicrobial agents. Intrinsic resistance can be attributed to its low permeability of outer membrane ,Multidrug Efflux system (For example, Mex AB- Opr M efflux system overexpression can lead to resistance to non- β lactams such as fluoroquinolones, sulfonamides, aminoglycosides and macrolides.) and the naturally occurring chromosomal AmpC blactamase.The major mechanism of acquired resistance to β -lactam antibiotics is through enzyme production that can inactivate beta-lactams and carbapenems such as extended spectrum beta lactamases (ESBLs) and metallo-β-lactamases (MBLs).Besides, exchangeable genetic elements such as plasmids, transposons and integrons are responsible for the dissemination of antibiotic resistance. Resistance gene cassettes in class 1 integrons 3 are associated with multi-drug resistance among Gram negative bacteria as well as *P. aeruginosa.* In addition, emergence of resistant mutants of Pseudomonas aeruginosa under antibiotic selective pressure during therapy allows them to be a formidable foe for burn care providers [2].

Multi drug resistance *P. aeruginosa* (MDRPA) is defined as an isolate intermediate or resistant to at least three groups of antibiotics among β-lactams, carbapenems, aminoglycosides, and Fluoroquinolones. Delay in providing appropriate treatment would lead to increased mortality and morbidity in terms of prolonged hospital stay, burn sepsis and graft rejection. Antimicrobial susceptibility pattern of Pseudomonas spp. isolated from burn wounds are continuously evolving. Outbreaks due to strains resistant to effective antibiotics, including carbapenem were reported elsewhere, efforts to monitor prevalence and resistant profile of MDR is very essential[3].

The lack of new antipseudomonal compounds in near future emphasizes the need for local surveillance of available agents against *Pseudomonas aeruginosa.*

This study was undertaken

1. To identify the isolation rate of MDRPA and its antibiotic profile.
2. To determine the MIC breakpoint of MDR -PA isolates to Ceftazidime.

MATERIALS AND METHODS:

This study was conducted over a period of 6 months in a burn unit of Government medical college Hospital and Research Institute, Chennai. Institutional ethical committee approval was obtained to conduct this study.267 consecutive nonrepititive samples were collected during this period.

Sample collection: After thorough cleaning with sterile saline all burn wound swab were collected aseptically. Three wound swabs were collected from patients admitted in burn unit, on Day 1, Day 4 and Day 10 respectively. In a leak-proof container specimens were transported to our Department of Microbiology for further processing.

Sample processing: Routine culture media like 5% sheep blood agar, Macconkey agar and Chocolate agar plates were inoculated and incubated overnight 37^{0}C aerobically. Pseudomonas was identified, isolated and speciated based on battery of biochemical tests by adopting standard microbiological techniques[4].

Determination of antimicrobial susceptibility by Disk diffusion method:

Antimicrobial susceptibility test by Kirby Bauer disk diffusion method recommended by Clinical Laboratory Standard Institute(CLSI)[5] was performed on all the isolates. It was performed on Muller Hinton Agar using the following panel of anti-pseudomonal antibiotics discs which were used to treat *P. aeruginosa* infections in our burn patients : Ceftazidime (30 μg), Piperacillin (100 μg), Piperacillin-Tazobactum (100/10 μg), Cefepime (30 µg), Gentamicin (10 μg), Amikacin (30 μg) and Ciprofloxacin (5 μg),Ofloxacin (10 μg) and Imipenem (10 μg).All antibiotic discs was procured from Himedia Ltd, India. Results were interpreted according to CLSI guidelines and these results were used for defining MDRPA. In our work, MDRPA was detected as a

bacterium which was Intermediate or resistant to three or more anti-Pseudomonal anti-microbial classes tested.

Determination of minimal inhibitory concentration by agar dilution method (MIC)[6]:

MIC of ceftazidime was determined by agar dilution method in accordance with CLSI document M07-A10..From a 10,000mg/L stock solution of Ceftazidime, appropriate working concentrations were prepared. 19ml of cooled molten agar was added to working concentration to achieve final concentration range of 256 to 0.25 µg/ml.Density of the inoculum is adjusted with saline to 0.5 McFarland standards. After overnight incubation at 37°C, inoculated plates were observed for MIC determination. The lowest concentration of ceftazidime that completely inhibits visible growth as judged by the naked eye, discarding a thin haze within the area of the inoculated spot was considered as MIC .The results were interpreted according to CLSI criteria for Pseudomonas spp. as follows:

Ceftazidime: Sensitive ≤8 µg/ml, Intermediate -16 & Resistant ≥32 µg/ml

Results: Out of 267 clinical samples, 108 *P. aeruginosa* was isolated. Antimicrobial susceptibility testing was carried out for all the 108 isolates of *P. aeruginosa* by Kirby-Bauer disk diffusion method. In this study MDRPA was defined as isolate, intermediate or resistant to three or more anti-Pseudomonal anti-microbial classes tested.

Among them, 38 was found to be MDRPA.Hence, the isolation rate was 35.18%.[Table 1]

Table1: Isolation rate of MDR Pseudomonas aeruginosa from burn wounds.

S.no.	Total no. of samples	Pseudomonas aeruginosa isolated(%)	MDR Pseudomonas aeruginosa isolated(%)	ISOLATION RATE (%)
1	267	108	38	35.18%

21(55.26%) of MDRPA isolates were associated with more than 50% total body surface area burnt (TBSAB), 10(26.31%) from 25.1% -50% TBSAB and 7(18.42%) from TBSAB less than 25%.

Analysis of MDRPA antibiogram revealed that 55% were resistant to beta-lactams – Piperacillin and 26% were resistant to piperacillin-tazobactam.A total of 19 isolates showed resistance (50%) to ciprofloxacin and 12(31.6%) to ofloxacin.

Table 2: Antibiotic profile of MDRPA from burn patients

S.no.	ANTIOBIOTICS	Sensitive	Resistant
1.	Piperacillin	17(45%)	21(55%)
2.	Piperacillin/tazobactam	28(74%)	10(26%)
3.	Cefepime	32(84.2%)	6(15.7%)
4.	Amikacin	23(60.5%)	15(39.4%)
5.	Gentamicin	12(31.6%)	26(68.4%)
6.	Ciprofloxacin	19 (50%)	19 (50%)
7.	Ofloxacin	26(68.4%)	12(31.6%)
8.	Imipenem	37(97.36%)	1(2.63%)
9.	Ceftazidime	0	38(100%)

Absolute resistance (100%). was observed for anti pseudomonal cephalosporins, Ceftazidime and Cefepime had least resistance 6(15.7%) comparatively. Among the aminoglycosides, 15(3.4%) isolates were found to be resistant to Amikacin and 26 (68.4%) to Gentamicin. 2.36% were resistant to carbapenem, Imipenem.[Table 2].

Isolates resistant to ceftazidime (100%) were subjected for MIC which revealed majority of the strains(18) had breakpoints at 64µg/ml,16 isolates at 32µg/ml and 2 strains at 128µg/ml.[Table 3]

Table 3: Distribution of MDRPA resistant to ceftazidime with reference to MIC

DISCUSSION:

Despite surgical advances in the management of burn wound infection, life threatening infections caused by MDR *Pseudomonas aeruginosa* has been frequently reported. In the present study, 40.45% (108/267) patients were found to be infected with *P. aeruginosa* during their hospital stay.

Among them, isolation rate of MDR *Pseudomonas aeruginosa* (38/108) was determined to be 35.18%.our findings are comparable to a similar study done in New Delhi, India, over a period of 2 years, where Biswal et al.,[7] observed 36.2%. In contrast, reports from Rajasthan in a recent study (2017)[8] observed 85.45% of isolates to be MDR. In various studies across the country, such as Upadhaya et al.,from Bangalore (2014)[9] and Puneet Bhatt et al., from Pune(2015)[10]reported 100% and 76.8% MDRPa respectively.

Analysis of isolates recovered from patients who sustained more than 50% BSA involved in burns revealed high isolation rate of 55.26% when compared to 18.42% of the strains with TBSAB less than 25%.Similar observation was reported by Keen et al., in his retrospective study[11]. This could probably explain the fact that mortality associated with large burns of Pseudomonas aeruginosa is due to its multidrug resistance.

On antimicrobial susceptibility testing, MDR strains were found to be highly susceptible to Imipenem (97.36%), followed by Cefepime (84.2%) and Piperacillin/Tazobactam (74%).

Third generation cephalosporin, Ceftazidime is still the recommended drug of choice in treating Pseudomonas aeruginosa infection. Even CLSI categorized them under group A but increasing resistance to this drug by MDR isolates is of great concern. All MDR-PA isolates in this study, was observed to be resistant to Ceftazidime(100%), which was supported by Wang et al., exhibited 100% resistance to this 3rd generation antibiotics[12]. Study reported by Hanza et al.,also explained Pseudomonas aeruginosa with 100% resistance to the same group[13]. In contrast, reports from developed country like U.S, revealed high sensitivity to Ceftazidime (95.5%) probably due to judicious use of this drug[14].

A notable observation was that 55% of the isolates which were found to be resistant to uriedopenicillin, Piperacillin demonstrated higher antibacterial activity when combined with betalactamase inhibitor Tazobactam (26%).This could be due to the production of extended spectrum betalactamases (ESBL) by these isolates.As ESBLs are inhibited by β-lactamase inhibitor concurrent administration expands its spectrum of activity.

Among the aminoglycosides(AMA), Amikacin has the least resistance (39.4%) compared to Gentamicin (68.4%). Javiya et al., also observed the same findings[15]. Amikacin appears to be promising; its use should be restricted for severe life threatening infections. Ofloxacin (68.4%) had better sensitivity compared to ciprofloxacin (50%) among the fluoroquinolones. Punnet bhatt et al., results are in accordance with this finding in a similar study[10].

Carbapenems are the antibiotics of choice for MDR P. aeruginosa infection but increasing resistance against carbapenems has now become a serious concern. Our study reported high sensitivity (97.36%) probably because of its judicious use in our settings.

Conclusion

Emergence of MDRPA infection in burn patient worldwide and unavailability of novel agents in near future, the effective measures at hand, would be stringent infection control measures. Active surveillance for MDRPA is of utmost importance to curtail the menace of antibiotic resistance posed by them.

Agent	Number of Ceftazidime resistant strains with MIC (µg/ml) n=38										
CAZ	0.25	0.5	1	2	4	8	16	32	64	128	256
No.	--	--	--	--	--	--	2	16	18	2	--

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