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Pseudomonas Aeruginosa Prevalence, Antibiotic Resistance and Antimicrobial use in Burn Wards from Nagpur City of Maharashtra

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Abstract: ***To assess the application of antibacterial agents, alongside pathogen, prevalence and Pseudomonas aeruginosa drug resistance, with the aim of understanding the impact of inappropriate antibacterial use. This retrospective study assessed bacteria from wounds, catheters, blood, faeces, urine and sputum of hospitalized patients in burn wards between June 2018 to December 2018. The intensity of use of antibacterial agents and resistance of P. aeruginosa to common anti - Gram - negative antibiotics were measured. Annual detection rates of Staphylococcus aureus were significantly decreased, whereas annual detection rates of P. aeruginosa and Klebsiella pneumoniae were significantly increased. Multidrug - resistant strains of P. aeruginosa were increased. The intensity of use of some anti - Gram negative antibiotics positively correlated with resistance rates of P. aeruginosa to similar antimicrobials. In burn wards, more attention should be paid to P. aeruginosa and K. pneumoniae. The use of ciprofloxacin, ceftazidime and cefoperazone/ sulbactam should be limited to counter the related increase in resistance levels.***

Keywords: antibacterial resistance, Pseudomonas aeruginosa, burn wards, drug usage, multidrug-resistant

1. Introduction

Although treatment for burns has been greatly improved, infection remains one of the main causes of death in burn patients, especially in critically - ill burn patients. [1–3] Indeed, compared with other hospitalized individuals, burn patients are characterized by skin deficiency, long hospital stays and multiple invasive operations, and are therefore more prone to infection. In addition, common bacterial species from burn patient wounds are constantly changing during the course of disease: initially, the burn wound is sterile, but it becomes colonized with Gram - positive bacteria such as β - haemolytic *Streptococcus* after 48 h. [4] With the application of surgical debridement and skin grafting in early surgery, as well as extensive use of systemic antibiotics and other treatment interventions, Gram - negative bacteria such as *Pseudomonas aeruginosa* can be detected. [5] During treatment, bacterial resistance also changes with the application of significant amounts of antibacterial agents. [6] Furthermore, bacterial prevalence differs between the burn wards of Gram - positive organisms. [8] Therefore, in the treatment for burns, regular monitoring of bacterial epidemiology in hospital wards is critical for the rational use of antibiotics. [9] different hospitals: some are dominated by Gram - negative bacteria, [7] while others predominantly report

In our burn ward, *P. aeruginosa* is the most prevalent bacteria, [10] and it is particularly difficult to treat. Indeed, *P. aeruginosa* harbours many virulence factors, including elastase, exotoxin A, phospholipase and homoserine lactone. [11] In addition, this organism possesses a variety of drug resistance mechanisms: inactivation or suppression of enzyme production, increased expression of an active efflux pump system, biofilm formation, and loss or decreased expression of outer membrane proteins. [12, 13] Therefore, multidrug - resistant (MDR) and extensively drug - resistant (XDR) strains are common. Burn patients infected with *P. aeruginosa* show a higher mortality rate. [14] Therefore, the development of effectively therapeutic strategies to treat *P. aeruginosa* infection has been the focus of our study group.

The widespread application of antibacterial agents has resulted in increasing levels and severity of bacterial resistance, [15, 16] which in turn, demands greater use of antibacterial agents, further aggravating bacterial resistance in a vicious cycle. [6] Thus, it is essential to select appropriate antibacterial agents, to avoid increased patient mortality[17] and the economic burden on patients and society. [18] However, in one study, more than 40% of antibacterial agents used in a hospital were reported to be inappropriate. [19] Similarly, a report from Tehran indicated that 40% of antibacterial agent use was inappropriate. [20] In the United States, irrational application of antibacterial agents has also been observed. [21] These deficiencies in the rational use of antibacterial agents are often accompanied by adverse consequences, including high mortality[22] and increased medical costs. [18] Therefore, it is not only necessary to monitor bacterial prevalence and drug resistance in hospital wards, but also antibacterial agent use. An increasing number of countries and researchers are now attempting to simultaneously monitor antibacterial agent use together with bacterial epidemiological data, with the aim of guiding policy development for the use of antibacterial agents. [23] However, many previous studies assessing anti - infective treatments for burns[7, 8] only monitored the prevalence and drug resistance of common bacteria in wards and neglected antibacterial use, making it difficult to understand the impact of inappropriate use of antibacterial agents in these cases.

In this retrospective study, we statistically analysed the use of antibacterial agents and bacterial epidemiology in wards treating burn patients. In particular, the use of antibacterial agents and drug resistance of *P. aeruginosa* were simultaneously evaluated, identifying any inappropriate antibacterial use. Through this combined analysis, we aimed to provide reliable data to guide policy development for the rational use of antibacterial agents in burn wards.

2. Materials & Methods

Bacterial sample collection

The study was retrospective. Bacterial samples were collected from hospitalized patients in the burn wards of Rural Hospital, of Nagpur from June 2018 to December 2018. A total of 96 hospitalized patients were enrolled, including 23 men and 48 women, and 25 children. Upon admission, the patients received routine preventative treatment which comprised lincomycin, and further treatment was adjusted according to antibiotic susceptibility test results.

Wound secretion specimens were collected for microbial culture at the first dressing change after admission, and subsequently on a weekly basis. Wound specimens were collected by sterile swabs from the wound surface after the removal of the dressing. In patients with central venous catheters, germiculture was also carried out with catheterization specimens and blood samples from ipsilateral/contralateral limbs when the catheter was extracted. In individuals with hyperpyrexia, diarrhoea, pulmonary infection (evidenced by a chest X - ray) and urinary tract infection, germiculture was also performed on blood, faecal, sputum and mid - stream urine samples, respectively.

Bacterial strain isolation and identification

All samples were routinely inoculated onto Mueller–Hinton agar medium procured by Hi Media Pvt. Ltd and incubated at 35°C for 24 h. After bacterial strain isolation and purification, identification was carried out using bacterial identification on a pseudomonas agar, specific biochemical test for identification of strains. Identical bacterial identification in different samples from the same patient indicated a positive result.

Drug susceptibility test: Drug susceptibility was determined by the Kirby–Bauer disk diffusionmethod. Antibiotic discs were procured from HiMediaLaboratories, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. [24] A total of six antibiotics were selected for assessing the drug resistance of *P. aeruginosa*, including amikacin (30 µg), ceftazidime (30 µg), cefoperazone/sulbactam (75/30 µg), imipenem (10 µg), meropenem (10 µg) and ciprofloxacin (5 µg). Standard strains for quality control were *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *Staphylococcus aureus*, which were all provided by the ATCC Centre for Clinical Laboratory. The results were expressed as the rate of resistant strains among all detected *P. aeruginosa* strains. MDR strains of *P. aeruginosa* were also calculated annually. The definition of MDR is resistance to three or more antimicrobial classes.

3. Results

From June 2018 to December 2018, a total of 174 pathogenic strains were isolated, including 43, 6, 8, 15, 24, 12, 18 and 66 from wound secretions, catheters, drainage fluid, throat swabs, blood, faecal and pus samples, respectively. The number of detected strains for each bacterial species, and the percentage of specific bacteria among all detected pathogens, were calculated.

Table 1: Pathogens detected from various samples isolated from burn patients.

Sr. No	Name of the bacterial strain	No of isolates	percentage
1	*Staphylococcus aureus*	46	26.43%
2	*Staphylococcus epidermidis*	27	15.51%
3	*Klebsiella pneumoniae*	25	14.35%
4	*Pseudomonas aeruginosa*	76	43.67%

Although *S. aureus* was the second predominant species during the work period, its percentage detection significantly decreased time to time. A similar decreasing trend was found for the detection of *Staphylococcus epidermidis*, with the rate reducing from 8.98%. By contrast, changes in the detection rates for *P. aeruginosa* and *Klebsiella pneumoniae* showed the opposite trend. The detection rate of *P. aeruginosa* significantly increased from 10.20% in July to to 26.16% till December, with this bacterium being the predominant species among Gram - negative bacteria during the work. . The detection rate of *K. pneumoniae* also significantly increased from 3.67% in to 12.25% in July to to 26.16% till December which was the third highest detection rate among Gram - negative bacteria. No significant changes in the detection rates were found for the other bacterial species assessed. Given that *P. aeruginosa* showed the most significant increase in the detection rate, our subsequent analyses focused on this bacterium.

From June 2018 toDecember 2018, the use of common antibiotics (vancomycin, penicillin, teicoplanin, imipenem, meropenem, lincomycin, minocycline, azithromycin, ciprofloxacin, cefradine, cefuroxime, ceftazidime, cefoperazone/ sulbactam and amikacin) in our department varied changes. The use of amikacin (mainly used against Gram - negative bacteria) and cephalosporins (used for both Gram - negative and Gram - positive bacteria), such as ceftazidime, cefoperazone/ sulbactam and cefuroxime, were significantly increased throughout the work. Specifically, the activity of amikacin increased from 8.65 to 49.41from June to December, and that of ceftazidime from 21.84 to 72.0. The values obtained for cefoperazone/sulbactam were 17.35 and 50.62 respectively and those of cefuroxime were 0.05. The increasing use of these antimicrobial agents targeting Gram - negative bacteria corroborated with the observed rise in the detection of Gram - negative bacteria, including *P. aeruginosa* and *K. pneumoniae*. The use intensities of the remaining antibiotics showed no significant increasing or decreasing trends.

Antibiotic sensitivity of the above antibiotics carried out in Muller Hinton agar and reported the changes occurred during the antibiotic susceptibility test. Results of these test were reported as below:

AkCefCezCefuImpe Mero TicarPipera

Table: 2. Changes in antibacterial activityused.

25.64	36.71	74.53	0.48	0.01	20.28	22.48	26.87
12.03	47.12	111.67	20.1	86.63	68.81	73.31	90.53
0	0	9.69	5.55	21.55	2.79	5.91	3.68
0.23	5.55	2.73	3.42	4.13	3.26	2.76	2.66
0.31	0	1.01	19.71	15.94	19.77	12.85	7.68

Changes in *P. aeruginosa* drug resistance

The resistance rates of *P. aeruginosa* to six common anti - Gram - negative antibiotics are shown including amikacin, ceftazidime, cefoperazone/sulbactam, imipenem, meropenem and ciprofloxacin. Interestingly, the resistance rates of *P. aeruginosa* to amikacin, cefoperazone/sulbactam, imipenem and meropenem were significantly increased. By contrast, the resistance rates of *P. aeruginosa* to ceftazidime and ciprofloxacin showed no significant trend but started to rise. These findings indicated that *P. aeruginosa* resistance to antibiotics targeting Gram - negative bacteria generally increased over the time period assessed. antibiotics.

The percentage (%) of *P. aeruginosa* resistant to specific antibiotics was measured by the Kirby–Bauer disk diffusion method and also the Changes in the prevalence of MDR strains of *P. aeruginosa*.

The number and percentage of detected MDR strains of *P. aeruginosa* were calculated at the end of the work, (Table 4). The percentage of MDR strains significantly increased fromJune 64.00% to 70% till the end of the study.

Table 4: Multidrug - resistant (MDR) strains of *P. aeruginosa* detected.

	Strains	%
June	16	64
July	24	58.54
August	29	64.44
September	57	78.08
October	68	80
November	64	95.52
December	57	86.36

We focused on *P. aeruginosa* because prevalence rates revealed a significant increasing trend, followed by *K. pneumoniae*, another Gram - negative bacterium. By contrast, Gram - positive *S. aureus* and *S. epidermidis* showed a significant decreasing trend in prevalence. This was in agreement with a recent study of pathogen prevalence and drug resistance in a burn ward, which reported 33.9%, 52.7% and 13.4% Gram - positive, Gram - negative bacteria and fungi, respectively. [26] Studies have reported that burn patients infected with Gram - negative bacteria, especially *P. aeruginosa*, [27] have a higher risk of death. Thus, more attention should be paid to Gram - negative bacteria, especially *P. aeruginosa* and *K. pneumoniae*, in determining antibiotic use in burn wards.

Our results revealed that the use intensities of amikacin, ceftazidime, cefuroxime and cefoperazone/sulbactam showed increasing trends. This might be due to extensive detection of Gram - negative bacteria, as these agents are commonly employed to treat Gram - negative bacterial infections. Although the intensity of use of vancomycin did not significantly increase, it remained high and ranked first for all of the months assessed This might be attributed to the fact that *S. aureus* always ranked second among the detected pathogens, although its rates of detection decreased over time.

It has been reported that *P. aeruginosa* strains detected in burn patients are usually MDR, i. e. show resistance to ciprofloxacin, cephalexin, aztreonam and ceftriaxone, [28] and are associated with higher mortality, [29] longer hospital stays and an increased number of ventilator days. [30] Patients with resistant *P. aeruginosa* infection have a poor prognosis and it is therefore increasingly important that close attention is paid to *P. aeruginosa* strains displaying severe drug resistance. Unfortunately, our study showed that the percentage of MDR *P. aeruginosa* strains in our burn ward had increased significantly from 64.00% to 70%. In this study, *P. aeruginosa* presented a significantly increasing trend in resistance rates to amikacin, cefoperazone/sulbactam, imipenem and meropenem. The resistance rates to ceftazidime and ciprofloxacin were also increased from 57% to 63%. Extensive use of antibacterial agents gradually leads to bacterial resistance. [15, 16] We speculate that the observed increased resistance rates may result from the continuous and significant overuse of these antibiotics; and for ceftazidime and ciprofloxacin, this overuse appeared to be relatively serious from 2010 to 2012 (Figure 2).

We further assessed the correlation between intensity of use of ceftazidime or ciprofloxacin, and the resistance rates of *P. aeruginosa*. Our results revealed that the intensity of use of ceftazidime was not significantly correlated with the resistance rates of *P. aeruginosa* to ceftazidime, and the intensity of use of ciprofloxacin was negatively correlated with the resistance rates of *P. aeruginosa* to ciprofloxacin. These findings do not contradict the association of drug resistance and use intensities of these two antibiotics because it may take time to increase *P. aeruginosa* resistance upon antibacterial overuse. For cefoperazone/ sulbactam, the intensity of use positively correlated with the cefoperazone/sulbactam resistance rate, indicating that resistance levels to certain antibiotics may increase without delay in *P. aeruginosa*.

The different timings for the appearance of drug resistance following drug overuse in *P. aeruginosa* may be attributed to the different antibiotic mechanisms. Drug resistance mechanisms in *P. aeruginosa* include: inactivating or inhibitory enzymes, increased active efflux pump system expression, changing target of antibacterial agents, biofilm formation, and loss or decreased outer membrane protein expression. [12, 13] Resistance mechanisms of *P. aeruginosa* to ciprofloxacin mainly include mutations in DNA gyrase and topoisomerase IV (encoded by the *gyrA* and *parC* genes, respectively). [31] It takes time for mutations to occur and spread within a bacterial population, which may account for the delayed drug resistance we observed. Exposure to ciprofloxacin may also increase expression of the active efflux pump system in *P. aeruginosa*, as a rapid stress response. Such resistance mechanisms often induce *P. aeruginosa* resistance to a variety of antibacterial agents. [13, 32] In agreement with this, we found that the intensity of use of ciprofloxacin was positively correlated with the resistance rates of bacteria to other antibiotics such as amikacin, cefoperazone/sulbactam, imipenem and meropenem. *P. aeruginosa* resistance to cefoperazone/sulbactam has likely increased active efflux pump system expression and biofilm formation. [33] Consistent with this, the intensity of use of cefoperazone/sulbactam was also found to be positively correlated with the resistance rates of bacteria to amikacin,

imipenem, meropenem and cefoperazone/sulbactam itself. Extensive use of ciprofloxacin or cefoperazone/sulbactam may thereby enable transformation of bacteria to MDR forms.

It is also worth noting that there are other sources of antibacterial agents. For example, antibacterial agents are sometimes added to foods, such as milk, [34–36] and it is conceivable that the regular consumption of such foods may contribute to antibiotic resistance. Therefore, in the face of serious levels of antibiotic resistance, [37–39] it is important to consider all possible contributory factors.

A few limitations of this study should be mentioned. First, this retrospective study only collected data for bacteria and antibiotic use from one hospital ward, and did not record the clinical characteristics and demographic features of patients, which might have impacted on bacterial resistance. Second, bacterial specimens were not subjected to molecular identification and homology analyses. Third, it was impossible to distinguish nosocomial from community - acquired infections, which might lead to excessive resistance rates. Furthermore, this was only a retrospective descriptive analysis, in which no control group was included, no intervention was applied for antibacterial agent use, and bacterial resistance variations were not analysed after intervention. These limitations should be considered when interpreting the data, and further studies are warranted to clarify these issues.

Based on our findings, we conclude that anti - bacterial treatment strategies in burn departments should focus on Gram - negative bacteria, especially *P. aeruginosa* and *K. pneumoniae*, for which the prevalence rates are increasing day by day. The use of ciprofloxacin, ceftazidime and cefoperazone/sulbactam should be limited to counter the increase in resistance of *P. aeruginosa* to these agents and other common anti - Gram - negative antibiotics. These findings also confirmed that it is insufficient to only monitor bacterial prevalence in burn wards when selecting appropriate therapy. Antibiotic use and the corresponding resistance status of bacteria must also be considered to ensure the rational use of antibacterial agents and the development of effective therapeutic strategies.

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