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RESEARCH ARTICLE

Occurrence of Extended Spectrum *Beta* - Lactamase Producing Enterobacteriaceae Causing Wound Infections

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ABSTRACT

At present scenario, the extended spectrum beta lactamase (ESBL) producing bacterial pathogen causes various life threatening infections lead to sepsis related mortality. A wide variety of microorganisms, like *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Enterobacteriaceae*-like *Klebsiella pneumoniae and Escherichia coli*, are involved. Extended-spectrum beta-lactamases (ESBLs) constitute a growing class of plasmid-mediated ß-lactamases which confer resistance to broad spectrum beta-lactam antibiotics. Resistance is generally increasing, with reports of multidrug-resistant isolates. This study was conducted to determine the ESBL producing bacterial isolates in our setting and describe their antimicrobial susceptibility. In the present investigation, 110 samples were screened on different bacteriological media. Wound samples were collected from different hospital of city. Antimicrobial susceptibility tests were done using disc diffusion technique as per the standard of Kirby-Bauer method. Bacterial pathogen identity was confirmed based on standard methods which included, Gram stain reaction, colonial morphology on media, lactose fermentation, catalase, oxidase, coagulase and indole tests. Out of 110 wound swab samples analyzed, 90 (87.8%) were culture positive. From 90 bacterial isolates, the bacteria were identified as *Pseudomonas aeruginosa* was the most frequently isolated organism 46.66%, followed by *E. coli* (24.44%), *Klebsiella spp.* (22.22%), *Proteus spp.* (8.88%) The antibiotic sensitivity test were shown that *Pseudomonas spp.* was mostly resistant to ampicillin (90.47%) and tetracycline (78.57%). *E.coli* showed the highest resistance to Ampicillin, Amikacin, cefotaxime and tetracycline were 90.90%, 86.36%, 81.81% and 72.72% respectively. *Klebsiella spp.* showed highest resistance to Ampicillin and gentamicin(83.33%), Amikacin, cefotaxime, cotrimoxazole showed 77.77% resistance. *Proteus spp.* had resistance rate of 100% to Ampicillin and Gentamicin. Amikacin and ceftriaxone showed 87.5% resistance to antibiotics. ESBL production among these isolates was checked by combination disc method and about 72% isolates were found to be ESBL producers. So our result indicates the fact that the physicians should be aware of this increasing resistance among our local clinical isolates and should change their therapy regime accordingly.

Keywords: extended spectrum beta lactamase, ESBL producing bacterial isolates, wound swab samples, disc diffusion technique, and Bacterial resistance.

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1. INTRODUCTION

All wounds are contaminated by both pathogens and body commensals ranging from bacteria and fungi to other parasites[1]. The gram negative aerobic rod is *Pseudomonas aeruginosa*. The facultative anaerobes include *Enterobacter* species, *Escherichia coli*, *Klebsiella* species and *Proteus* species. Burn wounds are highly susceptible to colonisation and infection and this is a major problem in the management of burn victims.Infected burn wounds are not only associated with a delay in epidermal maturation and deep scar formation, infected patients also tend to stay longer in the hospital and have a higher mortality rate due to sepsis when compared with non-infected patients.[2] Meanwhile, the organisms that would invade the tissue depend on the location of the wound. This is because of close correlations between microorganisms present in wounds in close proximity to those sites[3]. Wound infections are responsible for significant human mortality and morbidity worldwide. Wound infection results in sepsis, limb loss, long hospital stays and higher costs[4] *Pseudomonas aeruginosa* on its own has been of great threat in surgical site infection due to its records of staggering resistance to extended spectrum antibiotics.[5] Antimicrobial resistance can increase complications and costs associated with procedures and treatment. Antimicrobial resistance among pathogens of wound infections is on the increase.[6] The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to a greater incidence of infections caused by Enterobacteriaceae family. The widespread uses of antibiotics, together with the length of time over which they have been available have led to major problems of resistant organisms, contributing to morbidity and mortality.

Extended-spectrum β-lactamases (ESBLs) has emerged as an important mechanism of resistance in Gram-negative bacteria. β-lactam antibiotics are among the safest & most frequently prescribed antimicrobial agents all over the world in treating Gram positive and Gram negative infections.[7] Production of β-lactamases is the most common mechanism of the bacterial resistant for these antibiotics. These enzymes are numerous and are plasmid mediated, capable of hydrolyzing and inactivating a wide variety of β-lactam antibiotics. In addition, ESBL producing organisms exhibit co-resistance to many other classes of antibiotics resulting in limitation of therapeutic option. For this reason, the significance of such ESBL–mediated infections has been increasingly reported worldwide.[8]This study was done to determine the ESBL producing bacteria from burn wound isolates in our setting and describe their resistance patterns, which would enable the determination of empirical antibiotic strategies for the early treatment of imminent septic events.

2. MATERIALS & METHODS:

2.1. Collection of Samples:

Wounds samples were collected from different hospitals of Nagpur city. In this study, a total of 110 wound samples were collected in a sterile container. A swab taken from various aged patients with different locations of post operative wounds under sterile conditions. Dressed wounds were cleansed with non bacteriostatic sterile normal saline after removing the dressing. These samples were collected and transport to the laboratory within 4 hours for further screening.

2.2. Characterization and Identification of the Isolates :

The collected samples were streaked on freshly prepared nutrient agar plates and incubated at 37°C for 24 hours. Bacterial colonies differing in size, shape and colour were selected from the different plates and further subcultured on nutrient agar by the streak plate technique and incubated at 37°C for 24 hours after which, were maintained on agar slants for further characterization and identification. The bacterial isolates were characterized based on colonial and cell morphology, growth on differential/selective media such as Eosin Methylene Blue, CLED, Psuedomonas isolation agar, MacConkey Agar, Phenylalanine Agar etc. Biochemical tests which include Gram's reaction, indole tests, methyl red, Voges-Proskauer, Citrate Utilization, Motility, utilization of carbohydrates such as glucose, sucrose, mannitol, lactose and fructose, oxidase, catalase, coagulase and starch hydrolysis test[9]. The bacterial isolates were identified by comparing their characteristics with those of known taxonomy using the schemes of Cowan S. T.[10].

2.3. Susceptibility of antibiotics for ESBL production :

Antibiograms were carried out with Kirby- Bauer method. Disk-diffusion tests were carried out with antibiotic-containing disks on Mueller- Hinton agar plate. The results were expressed as susceptible or resistant according to the criteria recommended by the 'Clinical and Laboratory Standards Institute (CLSI). The following antimicrobial agents were tested: amikacin (30 µg), ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), gentamicin (10 µg), nalidixic acid (30 µg), tetracycline (30 µg), ceftizoxime (30 µg), and norfloxacin (10 µg) provided by Hi-Media, Mumbai.

2.4. Double Disc Diffusion method for ESBL :

Testing for ESBL production was carried out using Mueller Hinton agar plates that were inoculated with isolates. Separate commercial discs containing cefotaxime (30 µg),

ceftazidime (30 µg) and ceftriaxone (30 µg) with and without clavulanic acid (10 µg) were placed over the lawn culture. An increase in zone size of more than or equal to 5 mm for cefotaxime (30 µg), ceftazidime (30 µg) and ceftriaxone (30 µg),with and without clavulanic acid was considered to indicate ESBL producing strain as described by Carter *et al*. (2000).[11]

3. RESULTS & DISCUSSION:

In the present investigation, a total of 110 wound swab samples analyzed, 90 (87.8%) samples were culture positive (Table 1).

Sr. No.	Bacterial isolates n=90	Total number of isolates	Percentage
1.	*Pseudomonas* spp.	42	46.66
2.	*E. coli*	22	24.44
3.	*Klebsiella* spp.	18	22.22
4.	*Proteus* spp.	08	8.88
	Total	**90**	**100**

By *Pseudomonas aeruginosa* was the most frequently isolated organism (46.66%), followed by *E. coli* (24.44%), *Klebsiella spp.* (22.22%), *Proteus spp.* (8.88%) (Table No.1& Fig. 1).

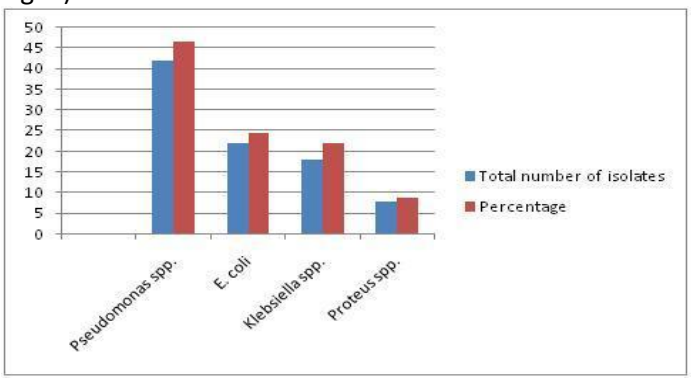

Fig. 1: Frequency of pathogens isolated from wound infections

This is similar to the observation in some other centres where *Pseudomonas spp, Klebsiella spp, E. coli* and Coliforms are the predominant pathogens responsible of wound and other nosocomial infections.[12] *Pseudomonas spp.* found to be the most common causative agent of wound infection, this study showed comparable results with the study of Mulugeta K. Azene (2011).[13] During the past decade, ESBL producing Gram-negative bacilli especially *Escherichia coli and Klebsiella pneumonia* have emerged as serious pathogens both in hospital and community acquired infections worldwide. The occurrence of ESBL among clinical isolates vary greatly worldwide and geographically and are rapidly changing over time.[14] Bacterial etiology can show geographic variations and may even vary over time within a population Difference in identification methods are also known to influence the relative prevalence of bacteria which makes comparison of results difficult.[15]

Analysis of species specific resistance rates indicated that most of *Pseudomonas spp.* was mostly resistant to ampicillin (90.47%) and tetracycline (78.57%). Also, *Pseudomonas spp.* was resistance to fourth generation antibiotics such as cefotaxime, ceftazidime, cotrimoxazole and ceftizoxime. *Pseudomonas spp.* was sensitive to norflaxocin, ciprofloxacin, and nalixidic acid with resistance of only 42.85%, 35.71% and 33.33%. This results showed some similarity with the study of Mulugeta K. Azene (2011)[13] showed *Pseudomonas* spp. had the highest resistance to tetracycline (90.3.0%) and Ampicillin. *E.coli* showed the highest resistance to Ampicillin, Amikacin, cefotaxime and tetracycline were 90.90%, 86.36%, 81.81% and 72.72% respectively. While *E.coli* was sensitive against ciprofloxacin and Norfloxacin. *Klebsiella spp.* showed highest resistance to Ampicillin and gentamicin(83.33%), Amikacin, cefotaxime, cotrimoxazole showed 77.77% resistance [Table No. 2 & Fig. 2].

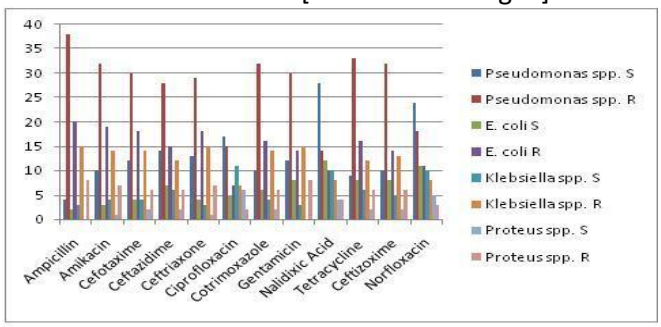

Fig. 2: Antibiotic susceptibility of pathogens isolated from wound infections

Significant resistance was seen in *K. pneumoniae* to many beta-lactam antibiotics in our study; a generally high level of resistance of Enterobacteriaceae to many antibiotics was noted in study of Brink A (2008). [16] *Proteus spp.* had resistance rate of 100% to Ampicillin and Gentamicin. Amikacin and ceftriaxone showed 87.5% resistance to antibiotics. All isolates were sensitive to ciprofloxacin, Norfloxacin and Nalidixic acid. High level of resistance has been reported to tetracycline from studies conducted in Ethiopia and elsewhere. [17] The sensitivity rates of norflaxocin, ciprofloxacin and nalidixic acid were high. Ciprofloxacin and norfloxacin were the most effective antimicrobials against most isolates. This finding is in line with the results documented from previous studies for ciprofloxacin (Anguzu & Olila, 2007),[18] and norfloxacin (Petkovs˘ek *et al.*, 2009).[19] Due to the nature of the retrospective analysis we couldn't trace patient's history. This study did not consider etiology of wound infections other than bacteria and anaerobic bacteria due to lack of facility. The number of antimicrobials were tested were also limited in some isolates. It is also recommended that in addition to using the above antimicrobial therapy in the treatment of wound infection, adequate attention should be placed on preventive measures such as hand washing, disinfection, good nursing practice and good surgical techniques amongst others, to reduce bacterial contamination of wounds.

Sr. No.	Antibiotics	Pseudomonas spp. n= 42			*E. coli* n=22			*Klebsiella* spp. n=18			Proteus spp. n=08		
		S	R	%R	S	R	%R	S	R	%R	S	R	%R
1.	Ampicillin	04	38	90.47	02	20	90.90	03	15	83.33	0	8	100
2.	Amikacin	10	32	76.19	03	19	86.36	04	14	77.77	1	7	87.5
3.	Cefotaxime	12	30	71.42	04	18	81.81	04	14	77.77	2	6	75
4.	Ceftazidime	14	28	66.66	07	15	68.18	06	12	66.66	2	6	75
5.	Ceftriaxone	13	29	69.04	04	18	81.81	03	15	83.33	1	7	87.5
6.	Ciprofloxacin	17	15	35.71	05	07	31.81	11	07	38.88	6	2	25
7.	Cotrimoxazole	10	32	76.19	06	16	72.72	04	14	77.77	2	6	75
8.	Gentamicin	12	30	71.42	08	14	63.63	03	15	83.33	0	8	100
9.	Nalidixic Acid	28	14	33.33	12	10	45.45	10	08	44.44	4	4	50
10.	Tetracycline	09	33	78.57	08	16	72.72	06	12	66.66	2	6	75
11.	Ceftizoxime	10	32	76.19	08	14	63.63	05	13	72.22	2	6	75
12.	Norfloxacin	24	18	42.85	11	11	50.00	10	08	44.44	5	3	37.5

Table No. 2 : Susceptibility patterns of bacterial isolates from wound infection

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Conflict of Interest: None Declared

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