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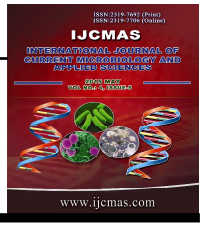

Original Research Article

Antimicrobial Susceptibility Pattern amongst Aerobic Bacteriological Isolates in Infected Wounds of Patients Attending Tertiary Care Hospital in Central India

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Wound infections, Gram positive bacteria, Gram negative bacteria, Antimicrobial susceptibility, Drug resistance, CRE

A B S T R A C T

Wound infection is one of the health problems that are caused and aggravated by the invasion of pathogenic organisms. Information on local pathogens and sensitivity to antimicrobial agents iscrucial for successful management of infected wounds. To assess the current status of bacterial pathogens involved in wound infections and their anti-microbial resistance patterns in a Tertiary Care Hospital. All pus/wound swabs were collected from July to September 2013 &were processed by routinely employed bacteriological conventional culture methods followed by Antibiotic Susceptibility Testing to find out Antibiotic susceptibility pattern amongst aerobic bacterial isolates. Out of 234(100%) samples 137(58.55%) showed growth & 97(41.45%) samples were found to be sterile after 48 hours of incubation. Out of 137 samples Gram negative bacilli (GNB) were 79(57.66%)& Gram positive cocci (GPC) were 58(42.34%). Among 79 GNB isolates *E.coli* was the commonest organism followed by *Pseudomonas aeruginosa* and *Klebsiella spp*. *Staphylococcus aureus* was the most common organism among GPC followed by *Enterococci.* Imipenam was the most sensitive drug for GNB isolates while Linezolid was the most sensitive drug among GPC. However, Carbapenem Resistance Enterobacteriaceae (CRE) among Gram-negative isolates was 19.57% in this study. This study shows that Carbapenem Resistance is on rise in Enterobacteriaceae (CRE) along with Non fermenters in tertiary care hospitals. So, antibiogram of bacterial isolates in wound infections is not only important for initiation of empirical treatment but also to practice the need for evidence based medicine & to avoid the misuse of antibiotics so as to prevent Multidrug Resistant Organisms (MDRO) in hospital settings in our efforts towards antibiotic stewardship.

Introduction

Wound is a breach in the skin and the exposure of subcutaneous tissue following loss of skin integrity caused by trauma, surgeries, burns, diabetic ulcers. Trauma may be accidental or intentionally induced. Wound provides a moist, warm and nutrient environment that is conductive to microbial colonization and proliferation that leads to serious bacterial wound infections and death.[1]Wound infections are one of the most common hospital acquired infections and are an important cause of morbidity and account for 70-80% mortality.[2]

Infection in wound constitutes a major barrier to healing and can have an adverse impact on the patient's quality of life as well as on the healing rate of the wound. Infected wounds are likely to be more painful, hypersensitive and odorous, resulting in increased discomfort and inconvenience for the patient.[3]

The current spread of multi-drug resistant bacteria pathogens has added a new dimension to the problem of wound infections. This is particularly worse in resource poor countries where sale of antibiotics is under poor control.[4]

Wound swab culture is the most frequently employed method of confirming wound infection. Properly performed wound swab cultures provide useful data to augment diagnostic and therapeutic decision making.[5]

A regular bacteriological review of infected wounds is therefore a necessity if affected patients must receive qualitative health care particularly when blind treatment is a necessity as in underdeveloped and developing nations.[6] This study is conducted to find out the various organisms causing wound infection and to identify the antibiotic susceptibility pattern of the isolated organisms.

Material and Methods

After clearance from the Institutional Ethical Committee a total number of 234pus samples were received after informed consent of patients for culture and sensitivity from different IPDs & OPDs in Microbiology Central laboratory during a period from July 2013 to September 2013.

The collected samples were processed for direct microscopy and aerobic culture and sensitivity as per the standard protocol. Samples were inoculated on Nutrient Agar, Blood Agar and Mac- Conkey Agar plates by streak plate technique and incubated at 37^0C aerobically for 24 hrs. Bacterial colonies were identified by standard microbiological tests.[7]Antimicrobial susceptibility of the isolates was performed on Mueller Hinton Agar plates by Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines.[8]

Data was compiles and statistical analysis was performed by using "Statistical Package for the Social Sciences" (SPSS) software - 16 and MS Microsoft Excel 2007.

Results and Discussion

Out of 234 pus samples received for culture and sensitivity in the Department of Microbiology 137 (58.52%) cases yielded positive culture while 97 (41.48%) cases had no growth. Among the 137 culture positive cases 75 (54.69%) were male and 62 (45.31%) were females (Table–1).

The Department wise distribution of culture positive pus samples revealed that culture positivity was highest in surgery department

41(29.93%), followed by Orthopaedics 26 (18.98%), ICU 22(16.06%), OPD 16(11.68%), Burn 12 (8.76%), Gynae & Obs. 10(7.30%), Medicine10 (7.30%) departments.(Table-2)

Among the 137 culture positive pus samples, 79 (57.66%) samples yielded Gram negative bacilli (GNB) and 58 (42.34%) samples yielded Gram positive cocci (GPC).

Out of 79 GNB isolates *E.coli*37 (27.01%) was the commonest organism followed by *Pseudomonas aeruginosa*28 (20.44%), *Klebsiella spp*. 9 (6.57%) and *Acinetobacter* 5 (3.65%). Amongst GPC *Staphylococcus aureus*46 (33.6%) was the most common organism followed by *Enterococci*7 (5.11%) and *Coagulase Negative Staphylococcus (CONS)* 5 (3.65%) (Table -3).

Most of the GPC isolates were sensitive to Linezolid while Imipenam was the most sensitive dru*g* among GNB. Among the *S. aureus* isolates, majority were sensitive for Linezolid (69.56%) and Piperacillin + Tazobactum (58.69%). Among *Enterococci* 85.71% isolates were sensitive to Linezolid & Piperacillin + Tazobactum While 100% isolates of CONS were sensitive to Linezolid & Piperacillin + Tazobactum.

As summarized in Table 5 most of *E.coli* isolates were sensitive to Imipenem (81.08%) & Polymixin-B (78.38%). Among the *P. aeruginosa* isolates, majority were sensitive forImipenem & Polymixin-B (78.57%). Among *Klebsiella spp* most sensitive drug was Imipenem(77.78) while Polymixin-B, Piperacillin+Tazobactum and Colistin were equally sensitive(66.67).

However *Acinetobacter spp* showed high sensitivity to Imipenem (100%) & Polymixin-B (80%).(Table-4 & 5)

The present study revealed that out of 234 samples 137 samples were culture positive. Among the 137(58.52%) culture positive cases 75 (54.69%) were male and 62 (45.31%) were females which corroborates with the study by Pappu A K et al.[9]This could because men are more prone for trauma because of their outdoor activities.

The Department wise distribution of culture positive pus samples revealed that surgery department had the highest incidence 41(29.93%), followed by Orthopedics 26 (18.98%), ICU 22 (16.06%), OPD 16(11.68%), Burn 12 (8.76%), Gynae & Obs. 10(7.30%), Medicine10 (7.30%) departments. Similar trend was also reported by Rao et al.[10]

Prevalence of Gram negative bacteria was more than Gram positive bacteria in our study. Findings are in consonance with a study conducted by Ghosh A et al(2009) in Kolkata.[11]

Staphylococcus aureus 46 (33.6%) was found to be highest incidence of all the isolates. This finding was similar with work done by Mahmood et al [12] and Nwachukwu et al[13].But it was contrary with work done by, Agnihotri N et al[14] which shows that *Pseudomonas aeruginosa* were showed highest incidence followed by *Staphylococcus aureus*.

Pseudomonas and *E. coli* were the most common Gram Negative Bacilli (GNB) isolated from the pus samples in our study. Such GNB dominance in the aerobic growth in pus culture has been highly seconded by studies reported by Ghosh A et al. [11]

The antibiogram of GPC showed highest susceptibility to Linezolid (74.12%) followed by Piperacillin /Tazobactam (64.51%), Clindamycin (56.90%) and

Levofloxacin (50.00%). Majority of Gram negative isolates were sensitive to Imipenam (81.01%) followed by Piperacillin+ Tazobactam (72.22%). Mahmood et al[12]also reported that the Gram negative isolates were found to be most susceptibility to Imipenam followed by Piperacillin+ Tazobactam.

Staphylococcus aureus showed the highest sensitivity to Linezolid (69.56%) and Piperacillin+ Tazobactum (58.69%). Devi et al also reported as *Staphylococcus aureus* were most susceptible to Linezolid (100%).[15]In this study 85.71% *Enterococci* isolates were sensitive to Linezolid, Piperacillin+ Tazobactam& clindamycin, these findings in consonance to study conducted by K Leela Rani et al[16] and Ajain et al.[17]*CONS*isolates were found to be susceptible to Linezolid (100%), Piperacillin +Tazobactam (100%) & Clindamycin. This was similar with the findings of devi et al.[15]

Mehta VJ et al[18] found in their study that Enterobacteriaceae isolates were 100% sensitive to Imipenam & 88% sensitive to Polymyxin B. Another study conducted by A Jain et al [17]showed 100% Enterobacteriaceae isolates were sensitive to Polymyxin B and Colistin followed by Imipenam (88.20%). However in our study, the members of Enterobacteriaceae family (*E. coli & Klebsiella*) showed high sensitivity to Imipenam (80.43%) followed by Polymyxin B (76.09%) and Colistin (73.91%).Carbapenem Resistance Enterobacteriaceae (CRE) among Gram-negative isolates was 19.57% in this study. However, overall CarbapenemResistance was about 30% reported by Henkhoneng Mate et al in their study.[19]

In this study among the non-fermenters (*Pseudomonas aeruginosa & Acinetobactor spp*) most sensitive drugs were Imipenam (81.82%) followed by Polymyxin B (78.79%) and Colistin (72.73%). However100% non-fermenters were sensitive to Polymyxin B, Colistin, Meropenem and Carbenicillinin a study conducted by Shanmugam Pet al.[20]These findings did not correlate with our study findings.

In present study GNB isolates showed significant resistance to higher antibiotics like Imipenam, Polymyxin-B&Colistin. The high rates of antibiotic resistance observed in the present study may be due to the widespread usage of broad spectrum antibiotics instead of evidence based medicine. Most of the patients before visiting a tertiary care hospital take earlier treatment from 'quacks', i.e. untrained people, who act as physiciansand start the treatment with higher antibiotics for quick relief.[21]This leads to development of Carbapenemase resistantEnterobacteriaceae (CRE) & this will further leads to resistance to Polymyxin B &Colistin.

Coinciding with the increased use of Carbapenems, multiple factors and modifiers likely contributed to the dramatic increase in CRE not restricted to urban area but started spreading to rural area. These include use of suboptimal or substandard antibiotics inhumans and animals, their relative penetration and selective effect on the gut microbiota, case-mix and infection control practices in different health care settings, and travel patterns.[22]Other risk factors for CREinfection includes hospital acquired infections following intensive care unit admission, severe illness, mechanical ventilation, prolonged hospitalization, etc.[23]

Limitation

Inappropriate use of antibiotics prior to specimen collection may have affected the

rate of the isolations. We have not done the confirmatory tests for detection of mechanism of drug resistance as it was a preliminary study identifying causative microorganism and their antibiogram test. Large scale multi-centric studies are required for substantiating the finding.

This study showed a preponderance of Gram negative bacilli over gram positive cocci in wound infection. In this study *S. aureus* was reported to be the commonest pathogen but the role of GNB, like *E. coli, Pseudomonas*, and *Klebsiella spp*. cannot be undermined. Wound infections are still a frequent & serious problem for many patients which can lead to increased morbidity, prolonged hospital stay and expensive treatment.Hence detection of pathogens from wound infections and their susceptibility pattern for commonly used antibiotics is essential.

This study also shows that Carbapenem resistance is high in our hospital. Hence rapid and accurate identification of Carbapenem resistance is required so that timely intervention, such as good infection control practices and evidence based use of antibiotics will ensure that the spread of Carbapenem resistance is kept under control. So, antibiogram of bacterial isolates in wound infections is not only important for initiation of empirical treatment but also to practice the need for evidence based medicine & to avoid the misuse of antibiotics so as to prevent drug resistance. Further, it is necessary that Health Education Programme and hygienic practices should be adopted in order to avoid community acquired spreading of wound infections causing bacteria.

Table.1 Sex wise distribution of culture positive pus samples

Sex	Culture positive (n=137)
Male	75 (54.69%)
Female	62 (45.31%)
Total	137

Table.2 Department wise distribution of samples

S.N	Ward	Culture positive Samples		
		GPC	GNB	Organisms isolated
		n (%)	n (%)	n (%)
1	**Surgery**	18(13.14)	23 (16.79)	41(29.93)
2	**Orthopedics**	15 (10.95)	11 (8.03)	26(18.98)
3	**ICU**	7 (5.11)	15 (10.95)	22(16.06)
4	**OPD**	6 (4.38)	10 (7.30)	16(11.68)
5	**Burn**	1 (0.73)	11(8.03)	12(8.76)
6	**Gynecology**	5 (3.65)	5 (3.65)	10(7.3)
7	**Medicine**	6 (4.38)	4 (2.92)	10 (7.3)
Total		58 (42.34)	79 (57.66)	137(100)

Table.3 Bacteria isolated (n=137) from pus culture

Catagory	Name of Organisms	Number (%)
GPC N=58	Staphylococcus aureus	46 (33.58)
	Enterococci	7 (5.11)
	CONS	5 (3.65)
GNB N=79	Escherichia coli	37 (27.01)
	Pseudomonas aeruginosa	28 (20.44)
	Klebsiella spp.	9 (6.57)
	Acinetobacter spp.	5 (3.65)
	Total	**137**

Table.4 Antibiotic Sensitivity Pattern of GPC(n=58)

	S. aureus (n=46) N (%)	**Enterococci (n=7) N (%)**	**CONS (n=5) N (%)**
Drugs	**S**	**S**	**S**
Ampicillin	12 (26.08)	4 (57.14)	3 (60.00)
Amoxyclav	21 (45.65)	5 (71.43)	4 (80.00)
Cefoparazone	22 (47.82)	5 (71.43)	4 (80.00)
Cefoxitin	8 (17.39)	4 (57.14)	3 (60.00)
Erythromycin	7 (15.22)	3 (42.86)	3 (60.00)
Gentamicin	16 (34.78)	4 (57.14)	3 (60.00)
Ciprofloxacin	10 (21.74)	4 (57.14)	3 (60.00)
Clindamycin	22 (47.82)	6 (85.71)	5 (100.00)
Amikacin	21 (45.65)	5 (71.43)	4 (80.00)
Cefepime	23 (50.00)	4 (57.14)	4 (80.00)
Levofloxacin	21 (45.65)	4 (57.14)	4 (80.00)
Linezolid	32 (69.56)	6 (85.71)	5 (100.00)
Piperacillin+Tazobactum	27 (58.69)	6 (85.71)	5 (100.00)
cefexime	16 (34.78)	5 (71.43)	4 (80.00)

Table.5 Antibiotic Sensitivity Pattern of GNB (n=79)

	Enterobacteriaceae (n=46)			**Non-fermenters (n =33)**		
	E.coli (n=37)	**Klebsiella spp (n=9)**	**Total (n =46)**	**P. aeruginosa (n=28)**	**Acinetobacter Spp. (n=5)**	**Total (n =33)**
	N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
Drugs	**S**	**S**	**S**	**S**	**S**	**S**
Amoxyclav	20 (54.07)	5 (55.56)	**25 (54.34)**	14 (50.00)	2 (40.00)	**16 (48.48)**
Ceftriaxone	15 (40.54)	4 (44.44)	**19 (41.30)**	10 (35.71)	2 (40.00)	**12 (36.36)**
Cefoparazone + Sulbactum	22 (59.46)	5 (55.56)	**27 (58.70)**	15 (53.57)	3 (60.00)	**18 (54.55)**
Tobramycin	23 (62.16)	5 (55.56)	**28 (60.87)**	15 (53.57)	3 (60.00)	**18 (54.55)**
Aztreonam	23 (62.16)	5 (55.56)	**28 (60.87)**	15 (53.57)	3 (60.00)	**18 (54.55)**
Cefpodoxime	19 (51.35)	5 (55.56)	**24 (52.17)**	17 (60.71)	3 (60.00)	**20 (60.61)**
Amikacin	21 (56.76)	5 (55.56)	**26 (56.52)**	14 (50.00)	3 (60.00)	**17 (51.51)**
Colistin	28 (75.68)	6 (66.67)	**34 (73.91)**	20 (71.43)	4 (80.00)	**24 (72.73)**
Levofloxacin	16 (43.24)	3 (33.33)	**19 (41.30)**	12 (42.86)	3 (60.00)	**15 (45.45)**
Imipenam	30 (81.08)	7 (77.78)	**37 (80.43)**	22 (78.57)	5 (100.00)	**27 (81.82)**
Polymyxin B	29 (78.38)	6 (66.67)	**35 (76.09)**	22 (78.57)	4 (80.00)	**26 (78.79)**
Piperacillin +Tazobactum	28 (75.68)	6 (66.67)	**34 (73.91)**	20 (71.43)	4 (80.00)	**24 (72.73)**

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