Original Research Article

Prevalence and Antimicrobial Susceptibility Patterns of Aerobic Bacterial Isolates from Pyogenic Wound Infections at a Tertiary Care Institute in Haryana, India

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A B S T R A C T

Wound infection is one of the health problems that is caused and aggravated by the invasion of pathogenic organisms. Information on local pathogens and sensitivity to antimicrobial agent is crucial for successful treatment of wounds. So the present study was conducted to determine antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to antimicrobial agents. The study was carried out from Jan 2015 to July 2015 in a tertiary care centre located in remote settings of Mewat. Wound swabs and pus samples were processed in the diagnostic microbiology laboratory immediately on reception following standard guidelines. The antibiotic sensitivity testing of all isolates was performed by Kirby Bauer's disc diffusion method and interpreted as per CLSI guidelines. Analysis of 438 pus samples showed 72.6% culture positivity with Surgical wards (39.7%) being the major contributor. Out of positive samples 82.3% were mono microbial and 17.7% poly microbial. *Staphylococcus aureus* (24.2%) was the most common organisms followed by, *Pseudomonas* (21.4%), *E coli* (14.8%), *Proteus spp* (8.8%), *Citrobacter spp* (8.2%), *Enterococcus* (6.6%), *Klebsiella spp* (6.1%) and *Streptococcus* (2.2%). Incidence of MRSA was 48.9% and 42.8% respectively. ESBL among *Enterobactericeae* were noted. Percentage resistance of isolates to individual antibiotic was also noted. The changing trends of antimicrobial susceptibility in bacterial isolates from pus can serve as a useful tool for physicians to start empirical treatment of patients at the earliest according to the geographical areas and emerging multi-resistant bacteria.

Introduction

Pyogenic infection is characterized by several local inflammation, usually with pus formation, generally caused by one of the pyogenic bacteria, which can produce the accumulation of dead leukocytes and infectious agent commonly known as pus. Pyogenic infections may be endogenous or exogenous .

A break or abrasion in the skin can provide an entryway for these surface bacteria into the body, and they stick very well to the moist edges of a cut. The bacteria begin to multiply and extend into the cut. The body's defense mechanism includes bringing immune cells into the area to fight against the bacteria. Eventually, accumulation of

these cells produces the thick whitish liquid that we call pus (Koneman *et al.,* 2005)

The inadvertent use of antibiotics leads to emergence of drug resistant pathogens, which in turn acts as a great challenge to the health services. Moreover, highly virulent strains and capacity to adapt quickly to changing environment worsens the situation and is a matter of concern (Sowmya N *et al.,* 2014). Skin and soft tissue infections that usually follow minor traumatic events or surgical procedures are caused by a wide spectrum of bacteria. Involvement of antibiotic resistant organisms in these infections increases the difficulty of their treatment and may have significant influence on the ultimate outcome.

Various studies across the globe have been consistent enough to show a predictable bacterial profile in the pyogenic wound infections. This makes an important observation for a clinician who intends to start empirical treatment to his patients, while laboratory culture reports are awaited. The present study was designed to evaluate the profile of aerobic pyogenic bacteria along with their susceptibility to antimicrobial agents in our region.

Materials and Methods

This prospective study was carried out in Department of Microbiology from January 2015 to July 2015. A total number of 438 pus samples were obtained for aerobic culture and sensitivity from different IPDs & OPDs of our hospital.

Pus samples were collected with sterile disposable cotton swabs and aspirates in syringe and were transported and processed in the microbiology laboratory immediately. They were inoculated on to Blood agar (BA), Mac Conkey agar (MA) and Nutrient agar (NA). Culture plates were incubated at 37°C for 24 hrs to 48 hrs in aerobic condition. After incubation, identification of bacteria from positive cultures was done using standard phenotypic methods. Simultaneously, a Gram stain was also performed on direct samples in which presence of pus cells and bacteria if present were noted. Also clinical details of patients were noted.

The antibiotic sensitivity testing of all isolates was performed by Kirby Bauer's disc diffusion method on Muller Hinton agar and interpreted as per CLSI guidelines and classified as sensitive and resistant (NCCLS. 2002.). Standard antibiotics ampicillin (30 μg), amoxyclav (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), gentamicin (10 μg and 120 μg), imipenem (10 μg), cefoxitin (30 μg), linezolid (30 μg), vancomycin (30 μg), piperacillin (100 μg), Ciprofloxacin (5μg), Erythromycin (10 μg), Netilmicin (30 μg), Colistin, Teicoplanin(30 μg), Amikacin (30 μg), Piperacillin Tazobactam (100/10 μg), Tigecyclin, Polymyxin B(10 μg) Clindamycin (2μg), Cotrimoxazole (Trimethoprim1.25μg/ Sulfomethoxazole 23.75μg) were tested. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 were used for quality control. All the culture media, biochemical media and antibiotics used were obtained from Hi Media.

Detection of Methicillin resistant Staphylococcus aureus (MRSA) was done with Cefoxitin (30 μg) disc. Extended spectrum betalactamase (ESBL) production was detected with the help of Ceftazidime (30 μg) and Ceftazidime + Clavulanic acid (30/10 μg). Results obtained were analyzed by counts and percentages using MS Excel, 2007 version.

Results and Discussion

A total of 438 samples were received during the study period out of which 318 (72.6%) were culture positive. Among these 262(82.4%) were monomicrobial i.e only one isolate obtained and 56(17.6%) were polymicrobial i.e culture yielded more than one isolate. The department-wise distribution showed that surgery (39.7%) department was the major contributor of pus samples followed by ENT (19.6%), orthopedics (18.3%), OBG (8.8%), ICUs (5.9%) and others(7.7%).The various bacterial isolates from different departments is shown in Figure 1.

A total of 364 organisms were isolated. The commonest organism were family *Enterobacteriaceae* (38.5%) followed by *Staphylococcus spp* (26.9%), *Pseudomonas* (21.4%), *Enterococcus* (6.6%), *Diphtheroids* (3.8%), *Streptococcus pyogens* (2.2%) and Acinetobacter (0.5%) as shown in Figure 2.

The most predominant gram positive bacteria (n=144) isolated was *Staphylococcus aureus* (61.1%) followed by Enterococci (16.7 %),CONS (6.9%), *Streptococcus pyogens* (5.6%) and Diphtheroids (1.4%). Though Enterobacteriaceae was the most predominant family isolated, *Pseudomonas* (35.5%) was the most predominant single Gram negative organism(n=220). Enterobacteriaceae (n=140) mainly comprised of *E.coli* (38.6%) followed by Proteus (22.8%), *Citrobacter* (21.4%), *Klebsiella* (15.7%) and *Morganella* (1.4%) as depicted in Figure 3.

Sensitivity of Gram positive bacteria were tested as per CLSI guidelines against Amoxycillin, Amoxy Clav, Cotrimoxazole, Ciprofloxacin, Erythromycin, Clindamycin, Gentamicin, Amikacin, Vancomycin,

Linezolid and Teicoplanin. Sensitivity pattern of Gram positive isolates is shown in Table1. Similarly Gram negative bacteria of family *Enterobacteriaceae* were tested as per CLSI guidelines against Ampicillin, Cefotaxime, Ceftazidime, Amikacin, Gentamicin, Imipenem, Ciprofloxacin, Colistin, Tigecyclin, Polymyxin B. Sensitivity pattern of family Enterobacteriaceae is shown in Table 2. *Pseudomonas* and *Acinetobacter spp* were tested against Ceftazidime, Amikacin, Gentamicin, Piperacillin, Netilmicin Piperacillin Tazobactam combination, Ciprofloxacin, Imipenem, Colistin, Tigecyclin and Polymyxin B as per CLSI guidelines. Their sensitivity pattern are shown in Table 3.Sensitivity pattern of Family Enterobactericeae and *Pseudomonas* and *Acinetobacter* is shown in Table 2 and Table 3 respectively.

MRSA was detected with the help of Cefoxitin (30 μg) and 43(48.9%) were found to be MRSA. ESBL producers among *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* was detected with ceftazidime (30 μg) and ceftazidime clavulanic acid (30/10 μg) disc and was found to be 18 (33.3%), 9 (40.9.1%) and 9 (28.1%) respectively.

Despite advances in the operative techniques and better understanding of the pathogenesis of wound infection, postoperative wound infection continues to be a major source of morbidity and mortality for patients undergoing operative procedures. Wound is a major concern among healthcare practitioners, not only in terms of increased trauma to the patient but also in view of its burden on financial resources and the increasing requirement for cost effective management within the health care, system (Bowler *et al.,* 2001). Its rate varies in different countries, different areas and even

in different hospitals. In present study, rate of wound infection was high, being 72.6%. Similar trends were reported by other studies conducted in India (Suchitra *et al.,* 2009) and (Manikandan *et al.,* 2013)

Though family Enterobacteriaceae was the most predominant group in our study, it was *Staphylococcus aureus* which was the predominant organism isolated and constituted 24.2% of the isolates. This was consistent with other similar studies (Swati Duggal *et al.,* 2015), (Tiwari *et al* .2010) and (Lee C Y *et al.,* 2009). Pseudomonas (29.73%) is the most common gram

negative bacterial isolate which is in accordance with the report of (Basu *et al.,* 2009). Surgical ward had given maximum number of pus samples followed by Orthopedic and ENT department.

The high prevalence of S. aureus infection may be because it is an endogenous source of infection. Infection with this organism may also be due to contamination from the environment e.g. contamination of surgical instruments. With the disruption of natural skin barrier *S. aureus*, which is a common bacterium on surfaces, easily find their way into wounds.

Table.1 Sensitivity Pattern of Gram Positive Isolates

Antibiotic	Staphylococcus spp (N= 98)	Enterococcus spp (N=24)	Streptococcus pyogens (N=8)
Amoxycillin	10 (10.2)	6(25.0)	8(100)
Amoxy Clav	21(21.4)	6(25.0)	8(100)
Cotrimoxazole	20 (20.4)	6(25.0)	8(100)
Cefoxitin	45(46.0)	-	-
Erythromycin	64 (65.3)	5(20.8)	8(100)
Gentamicin	70 (71.4)	-	-
Amikacin	75 (76.5)	-	-
Ciprofloxacin	22 (22.4)	-	8(100)
Clindamycin	74 (75.5)	8(33.3)	8(100)
Gentamicin 120	-	21(87.5)	-
Vancomycin	98(100)	24(100)	-
Linezolid	98(100)	24(100)	-
Teicoplanin	98(100)	24(100)	-

*Number in Parenthesis denotes Percentage

Figure.1 Department Wise Distribution of Bacterial Isolates
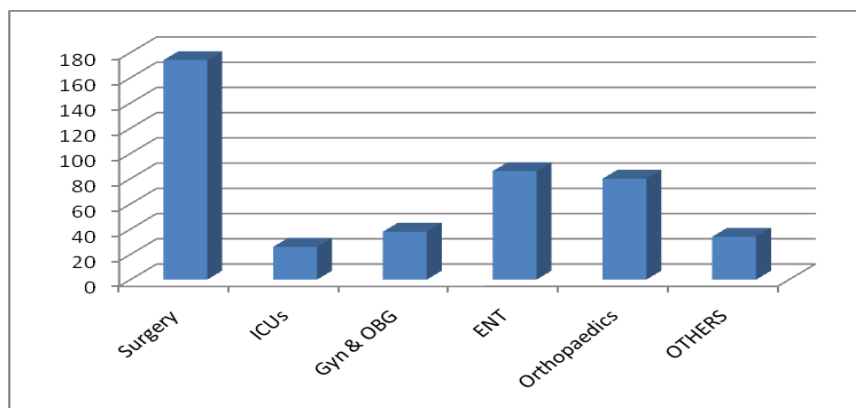
Table.2 Sensitivity Pattern of Enterobacteriaceae Isolates

| Antibiotic	E.coli (N=54)	Klebsiella (N=22)	Citrobacter (N=30)	Proteus (N=32)	Morganella (N=2)
Ampicillin	5(9.3)	2(9.1)	4(13.3)	0(0)	0(0)
Cefotaxime	27(50)	11(50.0)	19(63.3)	32(100)	2(100)
Ceftazidime	41(75.9)	14(63.6)	23(76.7)	28(87.5)	1(50)
Cefepime	42(77.8)	19(86.4)	21(70.0)	21(65.6)	2(100)
Gentamicin	29(53.7)	12(54.5)	20(66.7)	18(56.2)	1(50)
Amikacin	19(35.2)	13(59.1)	17(56.7)	14(43.7)	1(50)
Ciprofloxacin	6(11.1)	1(4.5)	10(33.3)	5(15.6)	2(100)
Piperacillin	-	-	-	-	-
Netilmicin	-	-	-	-	-
Piperacillin + tazobactam	-	-	-	-	-
Imipenem	54(100)	20(90.9)	30(100)	32(100)	2(100)
Polymyxin B	54(100)	22(100)	30(100)	32(100)	2(100)
Tigecyclin	50(92.6)	15(68.2)	29(96.7)	32(100)	2(100)
Colistin	54(100)	22(100)	30(100)	32(100)	2(100)

*Number in Parenthesis denotes Percentage

**Table.3** Sensitivity Pattern of *Pseudomonas* and *Acinetobacter spp*

Antibiotic	Pseudomonas (N=78)	Acinetobacter (N=2)
Ampicillin	-	0(0)
Cefotaxime	-	1(50.0)
Ceftazidime	61(78.2)	1(50.0)
Cefepime	-	-
Gentamicin	51(65.4)	1(50.0)
Amikacin	41(52.6)	1(50.0)
Ciprofloxacin	18(23.1)	0(0)
Piperacillin	5(6.4)	-
Netilmicin	1(1.3)	-
Piperacillin + tazobactam	26(33.3)	-
Imipenem	78(100)	32(100)
Polymyxin B	78(100)	-
Tigecyclin	-	0(0)
Colistin	78(100)	2(100)

*Number in Parenthesis denotes Percentage.

Figure.2 Percentage Distribution of Organisms Isolated
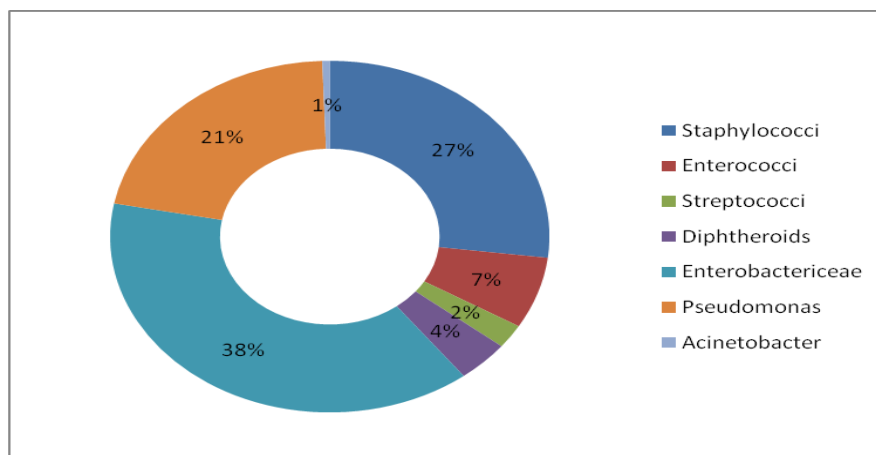
Figure.3 Distribution of Family *Enterobacteriaceae*
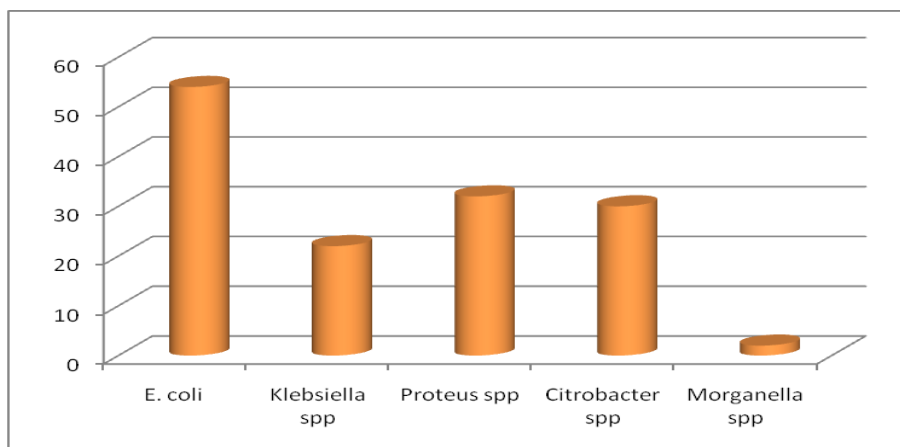
The antibiogram of gram positive cocci revealed that resistance to Vancomycin, Linezolid and Teicoplanin is yet to be seen in our set up. (Samra *et al.,* 2009) also have reported the same. *S. aureus* shows very high resistance to Amoxycillin, Amoxy Clav, Cotrimoxazole and Ciprofloxacin (sensitivity ranging from 10-30%).*S. aureus* was fairly sensitive to Amino glycoside group (sensitivity ranging from 70-75%). Also S.aureus was also sensitive to Macrolide group (sensitivity 60-70%). *Enterococcus* was highly resistant to Penicillins, Cotrimoxazole and Macrolides (sensitivity ranging from 20-30%). Senitivity to Gentamicin was good in *Enterococcus* (87.5%) and also no resistance reported in *Enterococcus* isolated from wound infection. *Streptococcus pyogens* was sensitive to all the drugs tested in our set up.

Gram negative bacteria of Enterobacteriaceae were most susceptible to Imipenem (sensitivity 90-100%), only two isolates of *Klebsiella spp* showed resistance. Enterobacteriaceae showed very high resistance to Ampicillin, Amoxy Clav and Ciprofloxacin (sensitivity ranging from 5-30%). Resistance to Cephalosporins ranged from no resistance to resistance in 50% isolates, where in *E.coli* and *Klebsiella spp* were the main contributors followed by

Proteus, *Citrobacter* and no resistance in *Morganella spp*. This finds concordance with (Balan *et al.,* 2013).Sensitivity to polymixin B and Colistin was 100% as all Enterobacteriaceae was sensitive. Sensitivity to Tigecyclin ranged from 68-100%, Klebsiella showing highest resistance as seven isolates were resistant.

No resistance against Imipenem, Polymixin B and Colistin was reported in *Pseudomonas* and *Acinetobacter spp* isolated from wounds (Table 3). Piperacillin and Netilmicin are no more effective against Pseudomonas as sensitivity is only 1-6%, Piperacillin Tazobactam combination being slightly better (sensitivity being 33.3%). Sensitivity to Amikacin and Gentamicin is still there in *Pseudomonas* being 52.6 and 65.4% respectively. Sensitivity to Ceftazidime is also good in our set up being 78%. This pattern of sensitivity of *Pseudomonas* and *Acinetobacter spp* has also been shown by (N.Sowmya *et al.,* 2014) and (HV Prashanth *et al.,* 2013). *Acinetobacter* is also still sensitive to Aminoglycosides and Ceftazidime whereas Tigecyclin has lost its effectiveness against *Acinetobacter spp* in our set up as none of the two isolate was sensitive to this drug.

In conclusion, Knowledge of the causative agents of wound infection in a specific geographic region is useful in the selection of antimicrobials for empiric therapy. As *S. aureus* was the most common organism isolated, it suggests that good hygine practices by the patient,their attendants and the health care providers is very crucial. Also fairly high rate of isolating *Pseudomona spp* needs attention. The susceptibility data suggests that multidrug resistance is not a severe problem in local area yet as we are a new set up. But rational use of antibiotics should be practiced to avoid emergence of multi drug resistant

strains. The in vivo susceptibility of the antimicrobials agents correlates well with the in vitro susceptibility reports and in our majority of the patients achieved a good therapeutic response.

Also the pathogen shows susceptibility to certain older drugs suggesting a cyclical wave pattern of susceptibility to antimicrobial agents. We recommend drug holidays for the group of drugs which have become ineffective over a period of time and bringing in the agents to which the organisms are sensitive. A changing trend in antibiotic sensitivity profile of the isolates need to be monitored as there is limited availability of newer drugs and the emergence of resistant bacteria far exceeds the rate of new drug development.

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