

Original Article

Aerobic bacteriological profile and antimicrobial susceptibility pattern of pus isolates from tertiary care hospital in India

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Abstract

Introduction: Pyogenic infections are an important cause of sepsis. These infections are difficult to treat because of the pathogens with increasing antibiotic resistance. It is important to know the pathogens causing the infections and its antibiotic susceptibility for proper management of the patients.

Methodology: A retrospective analysis of 1428 culture positive pus and tissue samples received in the department of microbiology from various departments in the hospital between January 2012 to 2017 was performed. Data regarding the pathogen isolated and its antimicrobial susceptibility were collected and analyzed. The specimens were primarily processed, as per standard methods. Identification and susceptibility testing was done using the Vitek-2C system.

Results: Among the samples males outnumbered females (M: F-2.5:1) and the median age was 47 years. The total number of patients were 1428 with total number of isolates being 1525 as in our study monomicrobial infections were seen in 93.2% (1331/1428) patients whereas combined infections with growth of two pathogens in 6.8% (97/1428). Gram-negative bacilli were isolated in 68.3% (1042/1525). Among the Gram-negative bacilli *Escherichia coli* was the major pathogen isolated (38.6%, 403/1042). Gram positive organisms were isolated in 31.6% (483/1525) of cases and *Staphylococcus aureus* was the predominant organism isolated (91.7%, 443/483). Rare pathogens like *Burkholderia pseudomallei* in 3 patients and *Nocardia* in one patient were also isolated.

Conclusion: This study emphasizes to understand the common organisms isolated from wound infections and it helps in empirical treatment of patients based on antibiotic susceptibility patterns.

Key words: pyogenic; multidrug resistant; abscess; melioidosis; *Nocardia*.

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Introduction

Pyogenic infections are an important cause of sepsis. The commonest organism causing wound infections was Staphylococcus aureus followed by other Gram-negative bacilli [1]. But in India Gramnegative bacilli was predominantly isolated compared to Gram-positive pathogens [2,3]. These infections are difficult to treat because of the pathogens with increasing antibiotic resistance [2]. The indiscriminate use of antibiotics has also lead to the increase in multidrug resistant organisms (MDRO) [3]. In the present era infections have become the leading cause of morbidity in patients of surgery, trauma etc. [4]. It is important to know the pathogens causing the infections and its antibiotic susceptibility for proper management of the patients [5]. The aim of our study is to determine the aerobic bacteriological profile from various type of wound infections and the susceptibility pattern of the isolates.

Methodology

A retrospective analysis of 1428 culture positive pus and tissue samples received in the department of Microbiology from various departments (orthopaedics, nephrology, plastic surgery, vascular surgery, surgical gastroenterology, cardiothoracic surgery) in the hospital between January 2012 to 2017 was performed. Demographic data regarding the pathogen isolated and its antimicrobial susceptibility were collected and analyzed.

Microbiological workup

The specimens were primarily processed, as per standard methods, on 5% sheep blood agar and Chromogenic agar (CPS ID) (bioMérieux, Marcy l'Etoile, France) and incubated at 37°C for 24 hrs. The Vitek2 (bioMérieux, Marcy l'Etoile, France) GN cards (ID GN panel) were used, for accurate identification of Gram-negative pathogens and Vitek 2 AST Cards (N281 panel), for antimicrobial susceptibility testing.

Table 1. Gram-negative bacilli isolated n = 1042 (68.3%).

Organism	Total	Percentage	Sensitive	ESBL	MDR
Escherichia coli	403	38.6%	82	257	64
Klebsiella pneumoniae	179	17.17%	12	86	81
Enterobacter cloacae	52	4.9%	42	-	10
Acinetobacter baumanii	97	9.3%	17	-	80
Pseudomonas aeruginosa	176	16.8%	101	-	75
Serratia marsecesns	47	4.5%	47	-	-
Proteus mirabilis	71	6.8%	71	-	-
Morganella morganii	13	1.2%	13	-	-
Total	1042		389 (37.3%)	343 (32.9%)	310 (29.7%)

Sensitive: Isolates that are sensitive to beta lactam/beta lactamase inhibitors, cephalosporins, aztreonam and carbapenems; ESBL: Isolates that are resistant to penicillin, cephalosporins and aztreonam; MDR: Isolates that are resistant to beta lactam/beta lactamase inhibitor combinations, 3rd generation cephalosporins, carbapenems.

The Vitek 2 GP cards (ID GP) were used, for accurate identification of Gram-positive pathogens, and Vitek 2 AST cards (P628) for antimicrobial susceptibility testing (AST).

Results

Demographic data

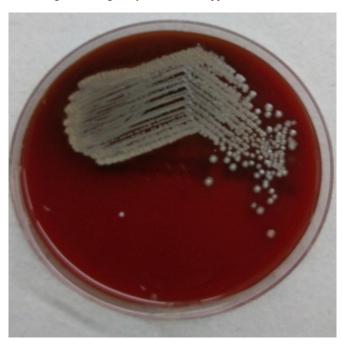
Among the samples males outnumbered females (M: F-2.5:1) and the median age was 47 years.

Microbial Spectrum of pathogens

The total number of patients were 1428 with total number of isolates being 1525 as in our study monomicrobial infections were seen in 93.2% (1331/1428) patients whereas combined infections with growth of two pathogens in 6.8% (97/1428).Gramnegative bacilli were isolated in 68.3% (1042/1525).Among the Gram-negative bacilli Escherichia coli was the major pathogen isolated (38.6%,403/1042). Other Gram-negative bacilli isolated were listed in Table 1.Gram-positive organisms were isolated in 31.6% (483/1525) of cases and Staphylococcus aureus was the predominant organism isolated (91.7%,443/483).Other Gram-positive cocci isolated were listed in Table 2.

Apart from the above organisms, rare pathogens like *Burkholderia pseudomallei* (Figure 1) in 3 patients and *Nocardia* (Figure 2) in one patient were also isolated. The total no of pathogens isolated from the year 2012-2017 are shown in Figure 3.

Figure 1. Colony morphology of *B. pseudomallei* on 5% sheep blood agar showing shiny carrom coin appearance.



Susceptibility pattern

Among the Gram-negative bacilli, 32.9% (343/1042) were Extended spectrum beta lactamase (ESBL) producers and majority of them were *E. coli* 63.7% (257/403). Multi drug resistance was observed in 29.7% (310/1042) of isolates among which 82.4% (80/97) of *Acinetobacter baumanii* were multi drug resistant (MDR) (Table 1).

Table 2. Gram positive bacilli isolated n = 483(31.6%).

Organism	No	Percentage 51.9%		
Methicillin sensitive Staphylococcus aureus	251			
Methicillin resistant Staphylococcus aureus	192	39.7%		
Streptococcus pyogenes	6	1.2%		
Enterococcus faecium	21	4.3%		
Enterococcus faecalis	13	2.6%		
Total	483	31.6%		

Figure 2. Colonies of *Nocardia* spp on blood agar showing raised dry chalky white colonies.



Figure 3. Total no of organisms isolated from 2012-2017 (excluding *B. pseudomallei* and *Nocardia*).

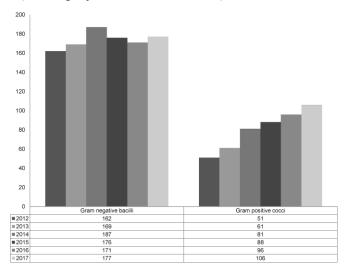


Figure 4. Antibiotic pattern of Gram-negative bacilli from 2012-2017 (excluding *B. pseudomallei*).

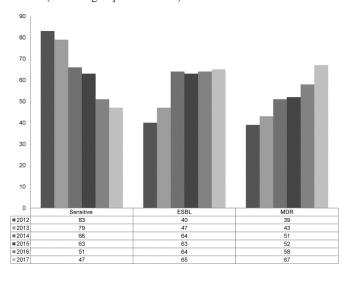
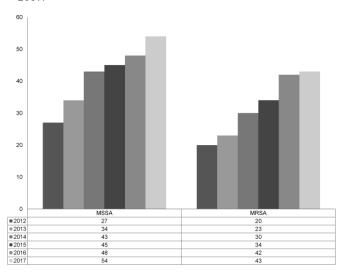


Figure 5. Isolation of *Staphylococcus aureus* from year 2012-2017.



Among Gram-negative bacilli, we found that there was a slight increase in ESBL and MDR isolates through the years. (Figure 4). There was also an increase in the total number of S. aureus throughout the years, but MSSA was predominantly isolated in comparison to MRSA. (Figure 5). There was no significant increase or decrease in the isolation of Enterococcus throughout these years. All pathogens were isolated from surgical site infections like intraabdominal surgeries, wound infections from trauma, abscesses like perianal abscess,intra-abdominal abscess, perinephric abscess, pyonephrosis and other skin and soft tissue infections.

Discussion

Pyogenic infections are characterized by inflammation with pus formation. These infections may be endogenous or exogenous [6]. Loss of skin integrity by various factors would provide an environment for the colonization and growth of microorganisms. The growth of the pathogens depend on the type of wound such as in clean wounds the growth would be minimal where as in traumatic wounds there would be an increased chance of infection requiring an aggressive management [7,8].

The infection may be monomicrobial or polymicrobial [6]. In our study 93.2% were monomicrobial infections whereas 6.8% were combined infections. Infections of wound can lead to increase in length of hospital stay and increased cost to the patients [9].

In a study in North India [10], they observed that 71.82% were Gram-negative and 28.18% were Gram-positive bacteria from wound infections and 63.2% of them were MDR [10]. In another study conducted in Nepal [11] Gram-negative bacteria were the predominant organisms isolated from wound infections. In our study Gram-negative bacilli was predominant accounting for 68.3% as most of our samples were from Intra-abdominal wounds which would be colonized by the gut flora.

Among Gram-negative bacilli, *E. coli* was the predominant organism isolated from pus samples as in seen in several studies [2]. It is the most common Gramnegative bacilli isolated in post-operative infections [12]. There was no significant difference in the total number of Gram-negative bacilli isolated throughout the years from 2012-2017, but there was a decrease in the number of sensitive isolates and an increase in ESBL and MDR isolates among Enterobacteriaceae in our study. Antibiotic resistance is of increasing concern as it is transmitted among patients leading to further

difficulty in treatment. Most of our Enterobacteriaceae were from surgical site infections like intra-abdominal surgeries, abscesses like perianal abscess, intra-abdominal abscess, perinephric abscess and pyonephrosis, but as this was a retrospective study, the source could not be determined. *E. coli* being predominant in 38.6% of our cases and 68.3% (257/403) were ESBL producers. *K. pneumoniae* was isolated in 17.17% of cases and nearly half of them were multi drug resistant (45.2%, 81/179).

Pseudomonas aeruginosa was isolated from 16.8% of our cases. In our study 57.3% (101/76) were sensitive to most of the antibiotics and others were MDR. The organism is mostly found in chronic infections [13]. In our study it was found in long standing cases of nonhealing wound infections in cases of fractures, abscesses etc.

Wound infections caused by *A. baumanii* are becoming more common. It is a hospital acquired infection and the organism is present in the environment. It is a colonizer in the skin and may later lead to infections [14]. *A.baumanii* was isolated from post- operative wound infections. 82.4% (80/97) were multidrug resistant and requires strict control over use of antibiotics and infection control measures.

In our study there was an increase in the number of Gram-positive cocci throughout the study period which was mostly due to the increase in the infections due to S. aureus. In a study from Bangladesh S. aureus is the commonest organism isolated in 40.5% of the cases of wound infections [1]. It is the commonest organism in most of the studies [15-17]. In a study by Bowler et al S.aureus is the most common cause of cutaneous abscess in 25-30% of the cases and mostly associated with acute soft tissue infections. It is also associated with bite wound infections and diabetic wound ulcers. It is one of the causes of delayed wound infection [8]. In our study S. aureus was found in 91.7% (443/483) of Gram-positive bacteria. MSSA was found in 51.9% and MRSA in 39.7% of the Gram-positive bacteria. All our Methicillin Resistant Staphylococcus aureus (MRSA) isolates were sensitive to vancomycin. Most of our patients were having soft tissue abscesses and ulcers and were treated on outpatient basis. Our patients with Methicillin-sensitive Staphylococcus aureus (MSSA) infections were treated with Tab Cloxacillin 500mg orally every 6hrs.MRSA infections were treated with intravenous vancomycin 1g 12th hrly or teicoplanin intravenously or Tab Linezolid 600mg 12th orally. Daptomycin 4mg/kg intravenously was given in severe soft tissue infections.

Other Gram-positive cocci like *Enterococcus* faecalis (4.3%) and *Enterococcus* faecium (2.6%) isolated in our study were seen in combined infections mostly in cases of wound infections in intra-abdominal surgeries. In a study from North India they isolated 86 isolates of *Enterococcus* and they found that *E. faecium* accounted for 56% of the skin and soft tissue infections [18]. Enterococci was treated based on the susceptibility pattern.

S.pyogenes were isolated from 6 cases in our study, 3 cases of chronic osteomyelitis, 2 cases of cutaneous abscess. In one case of necrotizing fasciitis, the organism was isolated from blood culture also in which the patient could not be revived. It is one of the frequent causes of delayed wound healing [8]. All our isolates were sensitive to penicillin, cephalosporins.

Combined infections were seen in 6.8% of our cases. Most of them were from post-operative abdominal surgeries and chronic wound infections. Polymicrobial growth was observed in most of the studies in post-operative wound infections [12,19,20]. Post-operative care should be given properly to prevent infection with different organisms which would be difficult to treat due to increased resistance to antibiotics. Delay in wound healing results in increased length of hospital stay and increase cost for treatment to the patients [8].

In our study of six years (2012-2017), we found that though there was no change in the spectrum of organisms causing infections but the organisms were becoming resistant to antibiotics. This may be due to the transfer of resistance genes among them. Emergence of extended spectrum beta lactamase (ESBL) producing isolates has important clinical and therapeutic implications. Extended-spectrum betalactamases (ESBL) are enzymes that confer resistance to most beta-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam (CLSI guidelines). In a study they found that the prevalence of ESBL producing organisms was found 18% amongst which E. coli was 53.7%, K. pneumonia 14.8%, P. mirabilis 12.9% and others 7.4% [21]. The prevalence of ESBL-producing organisms in another study was found to be 48.27% [22]. A report from Pondicherry, India, showed that ESBL production was 81% in E. coli and 74% in K. pneumonia [23]. In our study 32.9% of our isolates were ESBL producers, the highest among E. coli followed by K. pneumoniae. In mild infections with ESBL producer beta- lactam/beta-lactam inhibitor combinations may be considered, while in severe infections carbapenems are considered as the drug of choice [24]. Our patients were treated with

cefoperazone sulbactum 1-2g/day 12th hrly or carbapenems depending on the antibiotic susceptibility patterns (AST).

Multidrug resistant (MDR) was defined as. resistance to more than three classes of antibiotics of the five classes of antibiotics like beta lactam/beta lactamase inhibitor combinations, 3rd generation cephalosporins, carbapenems, fluoroquinolones and aminoglycosides [20,25,26]. In this study we have considered MDR as those isolates that are resistant to beta lactam/beta lactamase inhibitor combinations, 3rd generation cephalosporins, carbapenems. In a study in 2016, they found that multidrug resistant E. coli was found in 31.6%, followed by *K. pneumoniae* 30% [25]. In our study multidrug resistant A. baumanii 82.4% followed by K.pneumoniae 45%. Colistin was used with success in treatment of infections with MDR organisms. Tigecycline can be used for treatment of complicated skin and soft tissue and intraabdominal infections with MDRO [24]. Our isolates were treated with a combination of colistin and meropenem. Colistin acts on outer membrane of cell wall and creates pores allowing the other drugs to enter into the bacterial cell. Meropenem has bactericidal activity and binds to PBP of cell wall and inhibits cell wall synthesis [26,27].

We isolated *B. pseudomallei* from 3 pus samples of pseudo aneurysm of descending thoracic aorta, [28] submandibular abscess [unpublished data], and ulcerating thigh abscess in a SLE patient (published data) [29]. All these patients were treated with I/V meropenem 1 g 8th hourly as initial therapy for 14 days. They were discharged with advice to continue with cotrimoxazole for 3 months. The clinical manifestations of melioidosis vary and may involve every organ. Acute Pneumonia and bacteremia are the most common manifestations associated with high mortality. Other genitourinary presentations include infection. suppurative parotitis, various forms of central nervous system infection, osteomyelitis and septic arthritis, intraabdominal abscess, necrotizing skin infection, mycotic aneurysms or pericarditis and corneal abscesses [30,31].

We isolated *Nocardia* from cutaneous lesions of a patient with disseminated Nocardiosis. Blood cultures of the same patients also showed growth of Nocardia. The isolate was identified as *Nocardia otitidiscaviarum* by MALDI TOF (Matrix Assisted Laser Desorption/Ionization - Time of Flight, Bruker Daltonic MALDI Biotyper). As *Nocardia otitidiscaviarum* is commonly resistant to trimethoprim sulfamethoxazole, patient was treated with intravenous ceftriaxone 2 g twice daily, intravenous amikacin 1 g once daily [32],

but due to financial constraints the patient left against medical advice (LAMA). The most common predisposing factor was organ transplantation followed by malignancies like leukemia, lymphoma and Acquired Immunodeficiency Syndrome (AIDS). In our patient, no history of any underlying condition could be found.

The risk factors associated with infection by MDRO were commonly age, sex, previous antibiotic therapy, previous hospitalization, increased length of stay in the hospital, patient comorbidities like immunosuppression, chronic liver disease, heart disease etc. and general medical condition [33]. There was a slight increase in the number of resistant organisms through the years in our study which may be due to the spread of the resistant genes among the organisms. As our Institute is a tertiary care center majority of the patients get admitted after being treated from outside hospitals where most of the patients had severe infections and they were treated with higher class of antibiotics in other hospitals which may lead to the growth of multidrug resistant pathogens. Proper control over usage of antibiotics and infection control measures starting from primary health care centers to tertiary levels would help in the control of infection with resistant pathogens.

The sensitivity pattern varies in different regions of the country so institutional antibiogram and formulation of antibiotic policy would help in empiric antibiotic therapy with a reduction in rate of infections.

Limitations of the study

As this was a retrospective study we couldn't look into the significant determinants such as the source of infection, the duration of hospital stay, and clinical outcome.

Conclusion

This study emphasizes to understand the common organisms isolated from wound infections and it helps in empirical treatment of patients based on antibiotic susceptibility patterns. Although wound infections cannot be eradicated completely, proper wound care, and its management and above all implementation of infection control measures by following strict hand hygiene practices, education about the spread of bacteria through contaminated hands and environment would lead to a decrease in infections with resistant organisms which would be a burden to both the hospital and the patient.

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Conflict of interests: No conflict of interests is declared.