Research Article

# Study on Antibiotic Susceptibility Testing against Pyogenic Organisms from Wound Infections

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**ABSTRACT**

Most treatment failures were related to the inappropriate initial antibiotic therapy with insufficient coverage of multidrug resistant pathogens, the rationale using for the combinations of antibiotics to cover the multi-drug resistant was Gram Negative and Gram Positive organisms. About 150 samples were collected and examined for this study. Bacterial strains were isolated and characterized by using various biochemical tests. By doing disc diffusion method by Kirby –Bauer, it was found that most of the isolates were resistant and some were affected by different antibiotics for both Gram Positive Cocci and Gram Negative Bacilli. The ability to inhibit the growth of bacterial isolates indicates the effective cure of antibiotics as an antibacterial agent depending on their antimicrobial activity efficacy in infection and low toxicity.

**Keywords:** Multidrug resistant, Gram Negative, Gram Positive, Kirby –Bauer.

## INTRODUCTION

The invasion of multiplication of microorganisms in or on the tissues of a host constitutes infection. Infection may be classified in various ways. Pyogenic infections refer to bacterial infection that leads to the production of pus. Antibiotics are the chemicals designed to treat these pyogenic bacterial infection. The advent of the antibiotics and resultant development of resistant strains of bacteria has introduced new types of pathogens significantly dangerous to man. The antibiotics are not curing all infection and there are dangers in using them indiscriminately. Sometime an antibiotic can develop the normal flora of the body, thus compromising the body's natural resistance and making it more susceptible to a second infection that is a microorganism resistant to the antibiotic. Pyogenic infections are characterized by several local inflammations, usually multiplication of microorganism. Pyogenic infection destroys neutrophil through release of leukocidines forming abscess which is marked as typical characterization of infection. Complications arising from cutaneous and soft tissue infection with *Staphylococcusaureus* are a major clinical problem owing to the high incidence of these infection and wide spread emergence of antibiotic resistant bacterial strain therefore leukocidines producing bacteria are usually referred to as pyogenic bacteria and variety of microorganisms that cause inflammation and suppuration are the pyogenic bacteria. This group includes great number of species which have now been differentiated in human body. The most common bacteria from the suspected patient with pyogenic infection include *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa*[1]. In 2007, it was reported that *Staphylococcus aureus* (45.1%), Coliforms (16.9%), *Proteus mirabilis* (11.3%), *Pseudomonas aeruginosa* (9.9%), *Klebsiella pneumonia* (7%) and *Enterobacter* Sp (2.8%) were isolated from the septic wounds from the patients who undergo surgical operation[2]. It is reported that out of 74 isolates *Staphylococcus aureus* (37.5%), *Pseudomonas aeruginosa* (27%), *Streptococcus pyogenes* (12.2%), *Klebsiella pneumonia* (9.64%) and *Escherichia coli* (14.9%) are found from the automobile accident wound [3]. The main aim of the study is to determine the antibiogram of the pyogenic pathogen.

## MATERIALS AND METHODS

**Sample collection**: Pus samples were randomly collected from the patient's infection site e.g.: surgery wounds, diabetic ulcer foot and other wound infections and then transferred aseptically using a sterile cotton swab. A total of 150 samples were collected from the outpatient and inpatient departments of the medical college Trivandrum and CSI karakonam medical college, Kerala. (Fig:1)

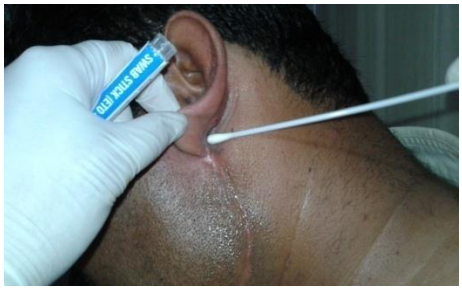

**Figure 1:** Collection of pus

### Isolation and Identification

The samples were inoculated for discreet colonies using a sterile wire loop following the standard procedure on the different media such as Mac Conkey's agar, Blood agar and Manitol salt agar and Brain heart infusion broth[4].

Microscopic examination was done by gram staining method. Confirmation of the isolated colonies was done with different biochemical tests (indole, urease, TSI, simmon's citrate, manitol motility, catalase, coagulase and oxidase). The entire tests were carried out by following the standard procedure[5].

**Antibiotic susceptibility test- Kirby Bauer disc diffusion method**

Isolated microorganisms were inoculated on Muller Hinton Agar. Antibiotic discs for the Gram Positive and Gram Negative were placed and incubated at 37ºC for 24h. The plates were observed to standard zone size of inhibition for Sensitive /Resistant against different antibiotics[6-8].

## RESULTS

A total of 150 specimens were processed from these131 were culture positives. Out of which, Gram Positive isolates were predominant (81%) followed by Gram Negative (18.9%). The most common organism isolated were *Staphylococus aureus* (66.41%),*Klebsiella* species (22.13%), Followed by *Pseudomonas aeruginosa* (11.45%)(Table 1).

*Staphylococcus aureus, Klebsiella* species and *Pseudomonas aeruginosa* were selected for this study because these are the commonly isolated pathogen from hospitalized patients.

**Table 1:** Organisms selected from pus culture

| S. no | Organism selected | Number of organisms isolated | Percentage |
|---|---|---|---|
| 1 | *Staphylococusaureus* | 87 | 66.41 |
| 2 | *Klebsiella species* | 29 | 22.13 |
| 3 | *Pseudomonas aeruginosa* | 15 | 11.45 |

***Staphylococcus aureus***

A common Gram-positive and Coagulase-positive organism which isthe part of the normal skin flora causes skin infections e.g. boils, abscesses, paronychia, pyaemia, osteomyelitis and wound infections. It is a frequent colonizer of skin and mucosa. They are the causative pathogen in nosocomial infections, MRSA has become a major problem in the medical and pharmaceutical industries and it is the common causative of nosocomial infections and has become resistant to the wide range of antimicrobial agents making it one of the more difficult pathogens to treat with conventional antibiotics [9, 10]. They are Non-motile, Gram-positive cocci, arranged in grape like clusters and produce pink colour colonies of mannitol salt agar ferment lactose, sucrose, glucose, and mannitol and produce acid. *Klebsiella* spp Frequently causing the hospital outbreaks of multidrug resistance pathogen. It's found in the normal flora of the mouth, skin, and intestines. A genus of aerobic, facultative anaerobic, nonmotile, non-spore-forming bacteria (family *Enterobacteriaceae*) containing gram-negative, encapsulated rods that occur singly, in pairs, or in shortchains.Theseorganismsproduceacetylmethylcarbinol andlysinedecarboxylase or ornithinede carboxylase. They do not usually liquefy gelatin. Citrate and glucose are ordinarily used as sole carbon sources.

***Pseudomonas aeruginosa***

It is a species that produces a distinctive blue-green pigment, grows readily in water, and may cause life-threatening infections in human which are difficult to treat due to multidrug resistance[11]. It is the bacteria which causes opportunistic infection in immune compromised patients. It is a species of gram-negative, non-spore-forming, motile bacteria that produce 'blue pus'. It is a non-lactose fermenting and do not ferment sugars.

**Antibiotic susceptibility test- Kirby Bauer disc diffusion method**

This study represents an attempt to determine trends in antimicrobial susceptibility patterns of the bacteria involved in Pyogenic Infections. In the study out of 150 samples 87.33 % were culture positives and 12.67% culture negative. The most common gram positive organism is *S. aureus*, which is sensitive to Gentamicin, Netilmicin, Cefazolin, Cephalexin, Cefuroxime, Vancomycin and Cloxacillin. In case of gram negative organisms, the *Klebsiella* sp show resistant to all the antibiotics such as Gentamicin, Netilmicin, Cefazolin, Cephalexin, Cefuroxime, Cefexime, Cefotaxime, Penicillin-G, Cotrimoxazole and Ampicillin. In case of *Pseudomonas aeruginosa*, it shows sensitivity to Ticarcillin and Piperacillin (Table 2).

## DISCUSSION

Pyogenic infection is characterized by severe inflammation with formation of pus. The infection occurs due to the colonization of Pyogenic organisms in the infected area. Pyogenic infection is mainly caused by *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa*[1]. The present study is performed to determine the drug sensitivity pattern of the pyogenic pathogens. Out of 150 samples, the most predominant pathogens are selected and characterized. They are *Staphylococcus aureus* (66.41%), *Klebsiella species* (22.13%), and *Pseudomonas aeruginosa* (11.45%) (Table 2). The susceptibility pattern of *Staphylococcus aureus* is highly sensitive to Vancomycin (94.25%), Cloxacillin (70.11%), Cefazolin (66.66%), and Cefuroxime (62.06%) and moderately sensitive to Gentamicin (56.32%), Netilmicin (50.57%), Cephalexin (52.87%), Cefotaxime(43.67%). It is resistance to penicillin-G (2.29%), Cotrimoxazole (36.78%) and Ampicillin(1.14%). It was reported that the *Staphylococcus aureus* isolates from the septic post operative wounds show sensitivity to Gentamicin (87.5%), Methicillin (75%), ciprofloxacin (68%)

and resistance towards Ampicillin. In case of MRSA, it is resistant towards Erythromycin (55%) and Methicillin(25%) [2]. A previous investigation on Community-acquired skin infection reveals that the antibiogram of *Staphylococcus aureus* shows high sensitivity to Vancomycin (80.5%), Methicillin (68%); moderately sensitive to erythromycin (55.6%), Novobiocin (54.1%) and resistance towards Bacitracin (25%) [12]. Among the isolates, the most frequently occurring gram negative pathogens are *Klebseilla* species and *Pseudomonas aeruginosa*. The susceptibility pattern of *Klebseilla* species show higher resistance towards Gentamicin (62.06%), Netilmicin (58.62%), Cefazolin (72.41%), Cephalexin (89.65%), Cefuroxime (65.51%), Cefexime (72.41%), Cefotaxime (75.86%), Cotrimoxazole (82.75%), Penicillin- G (96.55%) and Ampicillin (96.55%). In case of *Pseudomonas aeruginosa,* it shows higher resistance to the antibiotics such as Gentamicin (93.33%), Netilmicin (66.66%), Cefazolin (86.66%), Cephalexin (93.33%), Cefuroxime (80.00%), Cefexime (86.66%), Cefotaxime (73.33%), penicillin- G (100%), Cotrimoxazole (86.66%), Ampicillin (100%) and moderately sensitive to Ticarcillin (60.00%) and Piperacillin (53.33%). The isolates from the automobile accident wound infection shows resistant to penicillin (80.4%), streptomycin (67%) and Gentamicin (71.6%). It shows high sensitivity to Ofloxacin (81.6%), Ciprofloxacin (75.8%), Pefloxacin (81%) and moderate sensitivity to Augmentin (46.2%) and Nalidixic acid (56.8%) [3]. A previous report reveals that the most common isolates from burn wound infection is *Pseudomonas aeruginosa*(55.0%) which shows resistance towards Ceftazidime (70%) and Cefotaxime(66%) followed by *Staphylococcus aureus*(19.29%)which shows resistance towards erythromycin (84%), Cotrimoxazole (80%) and Oxacillin (40%). Other gram negative pathogens show high resistance towards Cefotaxime (66%) and Gentamicin(60%) [13].

**Table 2:** Antibiotic Susceptibility Pattern of Pyogenic Bacterial Isolates in pus.

| Antibiotics | *Staphylococcus* | | *Klebsiella* | | *Pseudomonas* | |
|---|---|---|---|---|---|---|
| | %of S | %of R | %of S | %of R | %of S | %of R |
| Gentamicin | 56.32 | 43.6 | 37.93 | 62.06 | 6.66 | 93.33 |
| Netilmicin | 50.57 | 49.42 | 41.37 | 58.62 | 33.33 | 66.66 |
| Cefazolin | 66.66 | 33.33 | 27.58 | 72.41 | 13.33 | 86.66 |
| Cephalexin | 52.87 | 47.12 | 10.34 | 89.65 | 6.66 | 93.33 |
| Cefuroxime | 62.06 | 37.93 | 34.49 | 65.51 | 20.00 | 80.00 |
| Cefexime | - | - | 27.58 | 72.41 | 13.33 | 86.66 |
| Cefotaxime | 43.67 | 56.32 | 24.13 | 75.86 | 26.66 | 73.33 |
| Vancomycin | 94.25 | 5.74 | - | - | - | - |
| Cloxacillin | 70.11 | 29.88 | - | - | - | - |
| Penicillin- G | 2.29 | 97.70 | 3.44 | 96.55 | 0 | 100 |
| Cotrimoxazole | 36.78 | 63.21 | 17.24 | 82.75 | 13.33 | 86.66 |
| Ampicillin | 1.14 | 98.85 | 3.44 | 96.55 | 0 | 100 |
| Ticarcillin | - | - | - | - | 60.00 | 40.00 |
| Piperacillin | - | - | - | - | 53.33 | 46.66 |

R: Resistant S: Sensitive %: Percentage

In the studies, the Gram Positive organism *Staphylococcus aureus* shows resistance towards Cotrimoxazole, Ampicillin and penicillin-G and sensitive towards Vancomycin which correlated with previous studies. In case of Gram Negative organisms, *Pseudomonas aeruginosa* and *Klebseilla* species show resistance towards almost all the antibiotics used for those studies and some of them coincides with the earlier reports too. Nowadays the prevalence of multiple drug resistance strains is developed due to the frequent use of antibiotics which leads to the production of b- lactamases. It is produced by both gram positive and negative organism. Its major mechanism of resistance is to attack the amide bond in b- lactam ring of penicillin[14] cephalosporin and the production of ESBLs - Extended Spectrum of Beta Lactamases[15] which is capable of hydrolyzing and inactivating the wide variety of b-lactams including the third generation cephalosporins, penicillins and aztreonam. The ESBLs producing strains are *Klebsiella pneumonia, Klebsiallaoxytoca, Escherichia coli, Enterobacter Sp., Salmonella Sp., Morganellamorganii, Proteus mirabilis, Serratiamarcescens* and *Peudomonas aeruginosa*[16] and in Gram positive organism *Staphylococcus aureus* produce beta lactamases enzymes[17].

## CONCLUSION

The present study shows the increasing trend of drug resistance among the bacteria abusing Pyogenic Infections. Antibiotics are most commonly prescribed inappropriately and inadequately and have thus become one of the highly abused agents. Moreover, the emergence of multi drug resistant organisms limits the choice of appropriate therapy. Present situation needs an active interaction between a clinician and clinical microbiologist to minimize the spread of multi drug resistant strains in the hospitals and to ensure authentic treatment to the patients.

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