Aerobic Bacteriological and Antibiotic Susceptibility Profile of Pus Isolates from A Tertiary Care Hospital, Puducherry

A. Deboral, Namrata K. Bhosale* and S. Umadevi

Department of Microbiology, Mahatma Gandhi Medical College & Research Institute, Pillaiyarkuppam, Pondy-Cuddalore Main Road, Pondicherry - 607 402, India.

Abstract

The antibiotic resistance pattern was observed significantly in various geographical locations. Routine surveillance is therefore essential for constant monitoring of AMR rates in the clinically important pathogens. It is imperative to track the changing resistance pattern over time, to guide proper therapeutic strategies to combat infections due to drug-resistant pathogens. This study aims to highlights the distribution of aerobic bacterial isolated from pus samples, and their susceptibility to different antibiotics collected during 2017 (July to December) in a tertiary care hospital. Nearly 637 clinical pus samples were received during July to December 2017 to the Department of Microbiology, Tertiary care hospital, Puducherry. Bacterial identification was performed using standard conventional biochemical tests and antibiotic susceptibility was carried out according to CLSI guidelines 2017 on each one of the aerobic bacterial isolates from the pus samples. Among the isolates 76.5% were Gram-negative bacilli (GNB) as well as 23.5% were Gram-positive cocci (GPC). The most common bacteria isolated were Pseudomonas spp 24.88% (108 in 434), followed by Escherichia coli 21.66% (94 in 434), Staphylococcus aureus 19.82% (86 in 434) and Klebsiella pneumoniae 13.13% (57 in 434). Of the 86 (19.82%) Staphylococcus aureus isolates, 16 (18.40%) were MRSA. Pseudomonas aeruginosa was highly susceptible to the carbapenems and least susceptible to ciprofloxacin. Acinetobacter baumannii was the most resistant organism according to this study and showed the least susceptibility to ceftriaxone and maximum susceptibility to aminoglycosides. This study concluded that the Pseudomonas aeruginosa isolate was found to be a predominant in our clinical pus samples. Gram negative bacteria are more commonly associated with the pyogenic lesion that Gram positive. A high level of an antibiotic resistance was observed in most of our bacterial isolates.

Keywords: Pus, Antibiotic susceptibility, antimicrobial resistance, bacteriological profile

*Correspondence: namukb1234@gmail.com; +91 9500628647

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INTRODUCTION

Pus is one of the most readily recognizable signs of an infection. Also traditionally defined as laudable pus is the thick, white, odorless exudate formed by pyogenic bacterial infections. Both aerobic and anaerobic bacteria have been found in hospital acquired infections, especially post-operative wound infections resulting in substantial morbidity, prolonged hospital stay makes a layman to create an economic stress factors. Antibiotics are widely used for therapeutic and prophylactic purposes but their unselective use in humans and animals combined with improved worldwide connectivity has led to a surge in antibiotic resistance. Antimicrobial resistance (AMR) especially Gram-negative bacilli have emerged as a significant public health problem globally due to insufficient public health problems. Infections from resistant organisms are linked to increased mortality and economic costs. India faces one of the world’s greatest burdens of drug-resistant pathogens. The rate of resistance in Gram-negative organisms is higher than that of Gram-positives. High ESBL rates have been reported in *E. coli*, *K. pneumoniae*; increased colistin and carbapenems resistance in *K. pneumoniae* and high carbapenem resistance rates in *Acinetobacter baumannii* than in *Pseudomonas aeruginosa*. Among *Staphylococcus species* a high inducible clindamycin resistance was mostly observed in MRSA compared to MSSA. The resistance profile across different geographical locations varies significantly. Routine surveillance is therefore essential for constant monitoring of AMR rates in the clinically important pathogens. Antibiotic sensitivity pattern has need to be monitored regularly for appropriate treatment required for multi-drug resistant pathogens. This study aims to highlight the distribution of aerobic bacterial isolated from pus samples, and their susceptibility to different antibiotics collected during 2017 (July to December) in a tertiary care hospital.

MATERIALS AND METHODS

This Prospective study included 637 pus samples which were received in the Microbiology department during 2017 (July to December) at Mahatma Gandhi Medical College & Research Institute, Puducherry, India. Bacterial identification was performed using routine diagnostic tests viz., conventional biochemical (IMViC) and sugar fermentation tests to detect up to the level of species level. The antibiogram of all the isolates was determined using Kirby-Bauer’s disk diffusion technique on Mueller-Hinton agar according to CLSI 2017 guidelines. Antimicrobial agents tested were as follows: cephalosporin (cefoxitin, ceftazidime, and ceftriaxone); β-lactam/β-lactamase inhibitor (cefoperazone/sulbactam and piperacillin/tazobactam); carbapenems (imipenem and Meropenem); fluoroquinolones (ciprofloxacin); aminoglycosides (amikacin, gentamicin, tobramycin) and polymyxins (colistin and polymyxin B). Batch wise testing was made to check the Quality control (QC) for freshly prepared biochemicals as well as agar plates by using CLSI guidelines. For QC recommended bacterial strains were used viz., *Escherichia coli* ATCC 25922, and ATCC 25923 *Staphylococcus aureus*.

Statistical Analysis

The results obtained were analyzed using MS Excel, 2010 version, with counts, percentages and pivot tables.

RESULT

Socio-demographic characteristics of the study population

Among 637 patients, 263 (41.29 %) were aged from 40 to 60 years followed by 203 (31.87 %) patients ranges from 20 to 39 years, 104 (16.33%) cases were aged more than 60 years and remaining 67 (10.51%) patients are below 20 years of age. In our study population, Male (56.36%) was the predominant when compared to females (43.64%) which might be explained by the fact that men are mostly involved in outdoor activities and occupation which increases their likelihood of injury.

| Age group | Female   | Male     | Total |
|-----------|----------|----------|-------|
| <20       | 26 (9.32%) | 41 (11.42%) | 67 (10.52%) |
| 20-39     | 92 (32.97%) | 111 (30.92%) | 203 (31.86%) |
| 40-60     | 131 (46.95%) | 132 (36.77%) | 263 (41.29%) |
| >60       | 30 (10.75%)  | 74 (20.61%) | 104 (16.33%) |
| Total     | 279 (100%)  | 358 (100%)  | 637 (100%)   |

Table 1. Age & sex structure of the study population
Patient Department (OPD) and (88.85%) from In-Patient Department (IPD).

**Bacterial Culture results for pus sample analysis**

Of the 637 samples, 434 (72.81%) had bacterial profile from In-patient (IP - Ward) and out-patient (OPD) department, among these isolates, 332 (76.5%) were showed pathogenic various gram-negative bacilli and cocco-bacilli growth, 102 (23.5%) had Gram positive cocci viz., *S. aureus, Enterococcus and Streptococcus* sps. Remaining 203 (36.10%) cultures were sterile.

Table 2 describes about the distribution of bacterial isolates from pus and wound samples. Nearly, 108 (24.88%) Pseudomonas isolates were found most predominant in pus samples followed by *Escherichia coli* (21.66%), *Staphylococcus aureus* (19.82%) which includes MRSA (18.40%) and *Klebsiella pneumoniae* (13.13%). Occurrence of other isolates in descending order are *Acinetobacter* sp (7.83%), *Proteus mirabilis* (5.30%), *Citrobacter* spp (2.76%), *Enterobacter* spp. (0.69%), *Providencia* (0.23%).

![Bacterial Isolates from Pus/Wound Swabs](image)

**Fig. 1.** Distribution of Bacterial isolates from Pus/wound swabs

| No. | Name of the Isolate            | In-patient isolates (n=381) | Out-patient isolates (n=53) | Total (n=434) |
|-----|--------------------------------|----------------------------|-----------------------------|--------------|
| 1   | *Staphylococcus aureus*        | 73 (19.2%)                 | 13 (24.5%)                 | 86 (19.8%)   |
| 2   | *Enterococcus sps.*            | 9 (2.4%)                   | 2 (3.8%)                   | 11 (2.5%)    |
| 3   | *Streptococcus sps.*           | 5 (1.3%)                   | 0                          | 5 (1.3%)     |
| 4   | *E. coli*                      | 86 (22.5%)                 | 8 (15.1%)                  | 94 (21.6%)   |
| 5   | *K. pneumoniae*                | 52 (13.6%)                 | 5 (9.4%)                   | 57 (13.1%)   |
| 6   | *Enterobacter sps.*            | 3 (0.8%)                   | 0                          | 3 (0.7%)     |
| 7   | *Citrobacter sps.*             | 9 (2.4%)                   | 3 (5.6%)                   | 12 (2.8%)    |
| 8   | *Acinetobacter sps.*           | 31 (8.3%)                  | 3 (5.6%)                   | 34 (7.8%)    |
| 9   | *Pseudomonas aeruginosa*       | 91 (23.9%)                 | 17 (32.1%)                 | 108 (24.9%)  |
| 10  | *Proteus sps.*                 | 21 (5.5%)                  | 2 (3.8%)                   | 23 (5.3%)    |
| 11  | *Providencia sps.*             | 1 (0.3%)                   | 0                          | 1 (0.3%)     |
Table 3. Antibiotic susceptibility profile of the aerobic bacterial isolates from pus

| Antibiotic       | S. aureus | Enterococcus spp | β hemolytic | E. coli | K. pneumoniae | Citrobacter spp | Enterobacter spp | Proteus mirabilis | Providencia spp | Acinetobacter spp | Pseudomonas spp |
|------------------|-----------|------------------|-------------|---------|---------------|-----------------|------------------|-------------------|----------------|-------------------|-----------------|
| Ampicillin       |           | 54.5             | 80          | 21.3    | -             | -               | -                | -                 | -               | -                 | -               |
| Penicillin       | 53.4      | 63.6             | 80          | -       | -             | -               | -                | -                 | -               | -                 | -               |
| Amikacin         | -         | 84.0             | 78.95       | 83.33   | 100           | 82.6            | 100              | 52.9              | 75              | -                 | -               |
| Gentamycin       | 95.3      | -                | -           | 71.3    | 64.91         | 50.00           | 66.6             | 73.9              | 100             | 55.8              | 69.4            |
| Tobramycin       | -         | -                | -           | -       | -             | -               | -                | -                 | -               | -                 | -               |
| High level       | -         | 81.8             | -           | -       | -             | -               | -                | -                 | -               | -                 | -               |
| Gentamicin       |           | -                | -           | 95.3    | 71.93         | 75.00           | 100              | 79.6              | -               | 71.3              | -               |
| Clindamycin      | 88.3      | -                | -           | -       | -             | -               | -                | -                 | -               | -                 | -               |
| Imipenem         | -         | 75.5             | 73.68       | 58.33   | 100           | 78.2            | 100              | 50.0              | 83.3            | -                 | -               |
| Meropenem        | -         | 82.9             | 78.95       | 75.00   | 100           | 100             | 100              | 47.1              | 82.4            | -                 | -               |
| Piperacillin      | -         | 73.4             | 73.68       | 83.33   | 100           | 100             | 100              | 44.1              | 81.5            | -                 | -               |
| Tazobactum       |           | -                | -           | -       | -             | -               | -                | -                 | -               | -                 | -               |
| Ciprofloxacin    | 26.7      | 27.2             | -           | 28.7    | 49.12         | 33.33           | 66.6             | 39.1              | 100             | 41.2              | 51.8            |
| Cotrimoxazole    | 68.6      | -                | -           | 40.4    | 47.37         | 66.67           | 100              | 39.1              | 100             | 47.1              | -               |
| Cefoperazone-sulbactum | -         | -                | -           | 78.7    | 71.93         | 75.00           | 100              | 100               | 100             | 50.0              | 79.6            |
| Ceftiraxone      | -         | -                | -           | 21.3    | 42.11         | 58.33           | 33.3             | 56.5              | -               | 14.7              | -               |
| Cefaclorizime    | -         | -                | -           | -       | -             | -               | -                | -                 | -               | -                 | 71.3            |
| Cefoxitin        | 81.4      | -                | -           | -       | -             | -                | -                | -                 | -               | -                 | -               |
| Erythromycin     | 59.3      | 45.4             | 40          | -       | -             | -                | -                | -                 | -               | -                 | -               |
| Tetracycline     | 48.8      | 36.3             | 40          | -       | -             | -                | -                | -                 | -               | -                 | -               |
| Teicoplanin      | 95.3      | 100              | 100         | -       | -             | -                | -                | -                 | -               | -                 | -               |
| Vancomycin       | 100       | 100              | 100         | -       | -             | -                | -                | -                 | -               | -                 | -               |
| Linezolid        | 100       | 100              | 100         | -       | -             | -                | -                | -                 | -               | -                 | -               |
DISCUSSION

In this study, 11 bacterial pathogens were isolated from pus and wound swabs which includes *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Citrobacter* spp., *Enterobacter* spp., *Enterococcus* spp., *Streptococcus* spp. Proteus spp and *Providencia* spp. The majority of the specimen yielded gram-negative bacterial isolates (76.5%) which is superior to gram-positive bacteria (16.0%) and this has been seen in earlier studies. The most common isolate was *Pseudomonas aeruginosa*, which coincided with findings of Lockhart et al and Agnihotri et al. Various researchers found that the *S. aureus* yields a predominant growth nearly 40–60% were isolated from different wound infections. This difference in the distribution of bacterial isolates may be attributed to the differences in study design, type of lesion, geographical location and climatic conditions. Among the 86 (19.82%) *S. aureus* isolates, 16 (18.40%) were identified as MRSA due to the resistance of Cefoxitin drugs which acts as a surrogate marker. Almost similar findings were found in another study from Nepal that the most of the bacterial isolates in pus samples. An efficient infection control program was followed and this could be results of reduced rate MRSA infections. Highest rate of susceptibility (95-100%) was seen toward teicoplanin, vancomycin and linezolid among the Gram-positive cocci. Both *S. aureus* and *Enterococcus* spp has been demonstrated that least sensitivity was observed towards ciprofloxacin (26-27%) (Table 3). In the earlier studies, gram-positive bacteria were predominant in most of the wound infections, but in contrast to the present study, gram negative bacteria i.e. *P. aeruginosa* was found frequently. Hanumanthappa et al, from Ballari district that demonstrate yielded nearly 56% of positive culture, but it was low (68.2%) when compared to the present study. According to the results of antibiogram of the present study, *Pseudomonas aeruginosa* was highly susceptible to the carbapenems (Meropenem followed by Imipenem) and least sensitive to ciprofloxacin (Table 3). *A. baumannii* was the most multi-drug resistant organism according to this study and showed a least sensitive to ceftriaxone and maximum to aminoglycosides (gentamicin followed by amikacin). *E. coli* isolates yields maximum sensitive to amikacin followed by meropenem, while being least susceptible to ampicillin and ceftriaxone. *Proteus mirabilis* did not exhibit any resistance to meropenem, piperacillin-tazobactam and cefaperazone-sulbactam but showed maximum resistance to ciprofloxacin and cotrimoxazole. The *Providencia* spp. isolated in this study was a pan sensitive strain. *K. pneumoniae* was more susceptible to tested antibiotics compared to *Citrobacter* spp. Both species showed resistance to the group of cephalosporins. Enterobacter isolates were highly susceptible to most of the broad spectrum of antibiotics.

CONCLUSION

To conclude that the gram-negative bacilli is more predominant when compared to gram positive and mostly associated with pyogenic lesions. The bacteriological profile of wound infections was similar to children as well as adults. A level of resistance was increasing in most of the bacterial isolates, this may reflect due to poor antibiotic stewardship. For monitoring the changing trend, periodic review of the bacteriological profile and pattern of antibiotic sensitivity is extremely essential.

Limitations

Isolation of anaerobic bacteria could not be carried out in the study and due to resource constraints, we were unable to confirm our results using molecular analysis. A multicenter study including larger sample size would have increased the significance of this study.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTION

Research conception & design: Dr. NKB, Dr. US, Performing the experiments: Ms. DA, Data acquisition: Ms. DA, Dr. NKB, Dr. US, Data analysis and interpretation: Dr. NKB, Ms. D, Dr. US, Drafting of the manuscript: Dr. NKB, Ms. D, Critical revision of the manuscript: Dr. NKB, Ms. D, Dr. US.
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ETHICS STATEMENT
Not applicable.

DATA AVAILABILITY-
Data available in the Department of Microbiology, Mahatma Gandhi Medical College & Research Institute, Pondicherry. Could be provided if necessary to anyone.

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