

Bacteriological profile and antibiogram of the bacteria isolated from pus samples in a secondary care unit

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Abstract

The human pus sample is the major one received from the Microbiology laboratory for culture and to study the antimicrobial susceptibility. A total number of 615 pus samples received to examine the aerobic culture and sensitivity from different IPDs and OPDs of Secondary Care Hospital in Namakkal District were processed for microbiological profile study and antibiotic susceptibility test. Analysis of 615 pus samples showed 67.31% culture positivity (Male:Female ratio=1.30:1.00) with Surgical wards (58.94%) being the major contributor. *S.aureus* (25.30%) was the most common organism followed by *Enterococcus sp* (18.80%). Antibiotic sensitivity profile of Gram positive bacteria showed sensitivity towards Meropenem (100 %), Amikacin (90.7 %), Piperacillin (67.8 %) and the Gram-negative was highly susceptible to Meropenem (98.2 %) and Doxycycline (69.0%). A high prevalence of MRSA (50%) was observed. Bacterial DNA was extracted and 16S rRNA sequencing was sequenced to characterize the highly antibiotic resistant bacteria and determine taxonomic classification. *Pseudomonas aeruginosa* isolates were confirmed by 16s rRNA sequencing which were resistant to amikacin, chloramphenicol, penicillin- G, tetracycline and vancomycin. Given the surge of antibiotic resistance, an assessment has been carried out to examine the antimicrobial susceptibility of bacterial isolates from pus samples to choose appropriate antibiotics for prophylaxis and the treatment of infections.

Key words: Pyogenic infection, Pus, Microbial profile, Susceptibility, *S. aureus*

Introduction

An inflammatory response to infection in the tissue including skin, ear, tooth, brain, liver, abdominal tissues, cervix, etc., leads to the formation of pus/abscess formation. Pyogenic bacteria including *Staphylococcus aureus*, *S. epidermidis*, *S.pyogenes*, *Escherchia coli*, *Streptococcus pneumonia*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis* or non bacterial pus forming organisms such as fungi, virus, protozoa, arthropod or surgical site infections

are major contributors of abscess/pus formation. In order to combat pathogens, the immune system releases antibodies into the infected area (Abbas AK, Lichtman AH 2014). The more dominant organism in the pus was *Staphylococcus aureus* followed by *Streptococcus* species (Lee et al. 2018). This infection causes serious morbidity, prolonged hospitalization and becomes an enormous economic burden (Kobayashi and DeLeo 2018). Besides, antibiotic resistance among these pyogenic bacteria results in improper treatment and management of diseases. The increased prevalence of drug resistance including methicillin resistant strains of *S. aureus* and biofilm formation leads to treatment failures and community spread of these multidrug resistant bacteria. The drug susceptibility patterns/antibiogram of the bacterial strains isolated from pus/wound swab samples could be used as a benchmark for understanding and treating sensitive or resistant pyogenic bacteria.

Several studies have demonstrated the presence of numerous bacterial groups in the pus and abscess samples isolated from liver, brain, skin and abdomen (Dinda et al. 2013; Reyna-Fabián et al. 2016; Fan et al. 2017; Belbase et al. 2017; Sapkota et al. 2019). Antibiotic patterns of these bacterial groups show an increasing trend in the antibiotic resistance and multiple risk factors are attributed towards this pattern. Sporadic clinical reports can help the clinicians and policy makers to predict and limit the spread of antibiotic resistant pyogenic bacteria. Therefore, a study was conducted in a secondary care hospital in Namakkal District, India to study the changing trends in antimicrobial resistance in various pus isolates. Various predictors and risk factors associated with the antibiogram of the isolated pyogenic bacteria were understood.

MATERIALS AND METHODS

Sample collection

This is a prospective study in which a total number of 615 pus samples from different tissue sources were obtained from Inpatient Departments (IPDs) and Outpatient Departments (OPDs) of secondary care hospital in Namakkal District during the period of January-December 2018. Isolation of aerobic microbial culture and sensitivity pattern against commercially available antibiotics were studied to predict the antibiogram of the isolated strains.

Isolation of pus associated bacterial pathogens

The pus samples from patients were collected by sterile disposable cotton swabs and syringes, then transported and handled immediately under laboratory conditions. The pus samples were inoculated on Blood agar (BA), MacConkey agar (MA) and Nutrient agar (NA) medium. The culture plates were incubated for 24 hrs at 37°C. After isolation, the pathogenic strains were identified by conventional identification techniques such as catalase, coagulase, indole, methyl red, Voges-Proskauer, citrate, urease, phenyl pyruvic acid and oxidase test (Parajuli et al. 2014).

Antibiotic sensitivity test

The antibiotic sensitivity tests were achieved (Raza et al. 2013) on Muller Hinton agar and explained according to the CLSI procedure (Clinical and Laboratory Standard Institute, 2012) to identify the sensitivity pattern of the pathogenic bacterial strains isolated from the pus samples and grouped as sensitive and resistant. Drug sensitivity detection of MRSA isolates were performed

by using *Cefoxitin* disc (30 µg) and *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were used as control (Chakraborty et al., 2011). Amikacin (30 µg), Chloramphenicol (25 µg), Penicillin- G (2U/Disc), Tetracycline (30 µg) and Vancomycin (30 µg), Meropenem (10 mcg), Doxycycline (30 mcg), Gentamycin (5 mcg), Cefoperazone (75 mcg), Ciprofloxacin (5 mcg), Norfloxacin (10 mcg), Nitrofurantoin (300 mcg), Azithromycin (15 mcg), Ofloxacin(5mcg), Piperacillin-tazobactam (100/10 mcg) were used for determining antibiotic sensitivity of pyogenic bacteria.

Genomic DNA isolation and PCR

Antibiotic resistant *Pseudomonas* sp. isolates were homogenized with a FastPrep®-24 Instrument using Lysing Matrix-A (MP Biomedicals, USA). The DNA was extracted using high pure PCR template preparation kit (Roche, Germany) according to manufacturer instruction. The universal primers were synthesized (Raja et al. 2017) and the cycles were set with denaturation at 95°C for 2 min, 35 cycles of 95°C for 1 min, annealing at 52°C for 45 s and final extension at 72°C for 5 mins. All other procedures were followed as per our previous work (Raja et al. 2017).

Phylogenetic analysis of Antibiotic resistant *Pseudomonas* sp. isolates

Closely related sequences have retrieved from the NCBI database, and phylogenetic analysis has done using the Maximum Likelihood (ML) method. The tree with the highest log likelihood (-2494.36) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pair wise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 5 nucleotide sequences. There were a total of 1581 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018).

RESULTS AND DISCUSSION

Out of 615 pus samples obtained in IPDs and OPDs of secondary care hospital in Namakkal District and processed for aerobic culture and antibiotic sensitivity, 67.3 % (414) samples were positive with 32.68% (201) samples showed no bacterial growth (Fig. 1A). Among 414 samples, 234 (56.6%) were male patients and 180 (43.4%) were female patients (Fig. 1B) with a male: female risk ratio of 1.30:1.00. The department-wise distribution of pus samples positive (Fig 1D.) for aerobic culture showed that the surgery department (58.94 %) was a major contributor followed by OPD (14.01%), department of general medicine including eye, pediatrics (12.32 %), obstetrics and gynecology (OG: 11.11%) and trauma care ward (3.62 %) (Fig. 1C). Pyogenic bacteria isolated from the pus samples were identified based on the biochemical tests and isolated.

Majority of the pus samples showed the presence of the Gram positive bacteria *Staphylococcus* Sp. (25.12 %) followed by the other isolates such as *Enterococcus* (18.80%), *Proteus* sp (14.70%), *E. coli* (13.73%) and *Pseudomonas* Sp. (8.43%). Coagulase negative *Staphylococcus* (CONS: 4.11%) also contributed to collection of pyogenic bacteria. In our study, a Gram-negative bacteria was found to be the dominant causative of pyogenic abrasion which is supported by the previous study (Zubair et al. 2011).

Antibiotic resistance is a serious problem and a continuous examination is necessary to screen the susceptibility of these pathogens and to choose suitable regimens for prophylaxis and

treatment. Among all the antibiotics tested, meropenem and amikacin was shown to be effective in treating Gram positive and negative bacteria, in contrast to other antibiotics tested in this study. Similar studies to understand the antimicrobial sensitivity against bacteria isolated from various pus cultures in a tertiary hospital have been conducted (Tiwari and Kaur 2010).

S. aureus (25.30 %) is the most common Gram-positive isolate in our study as shown in previous studies (Lee et al. 2009; Tiwari and Kaur 2010). However, higher rates were reported by following studies (Kshetry et al. 2016) (37.6%), (Sanjana et al. 2010) (39.6%), (Dibah et al. 2014) (46.3%), and (Tiwari et al. 2009) (69.1%). The difference in the prevalence of MRSA in various studies might be due to unreasonable antibiotic used in the selection of pyogenic bacteria. *Staphylococcus* sp. was susceptible to Meropenem (100%) and Amikacin (90.7 %) similarly (Samra et al. 2005) reported 100% sensitivity. Antibiotic sensitivity profile of Gram-negative bacteria showed high sensitivity to Meropenem (98.2%) followed by Doxycycline (69.0%) and amikacin (65.7%) (Fig. 2A-B).

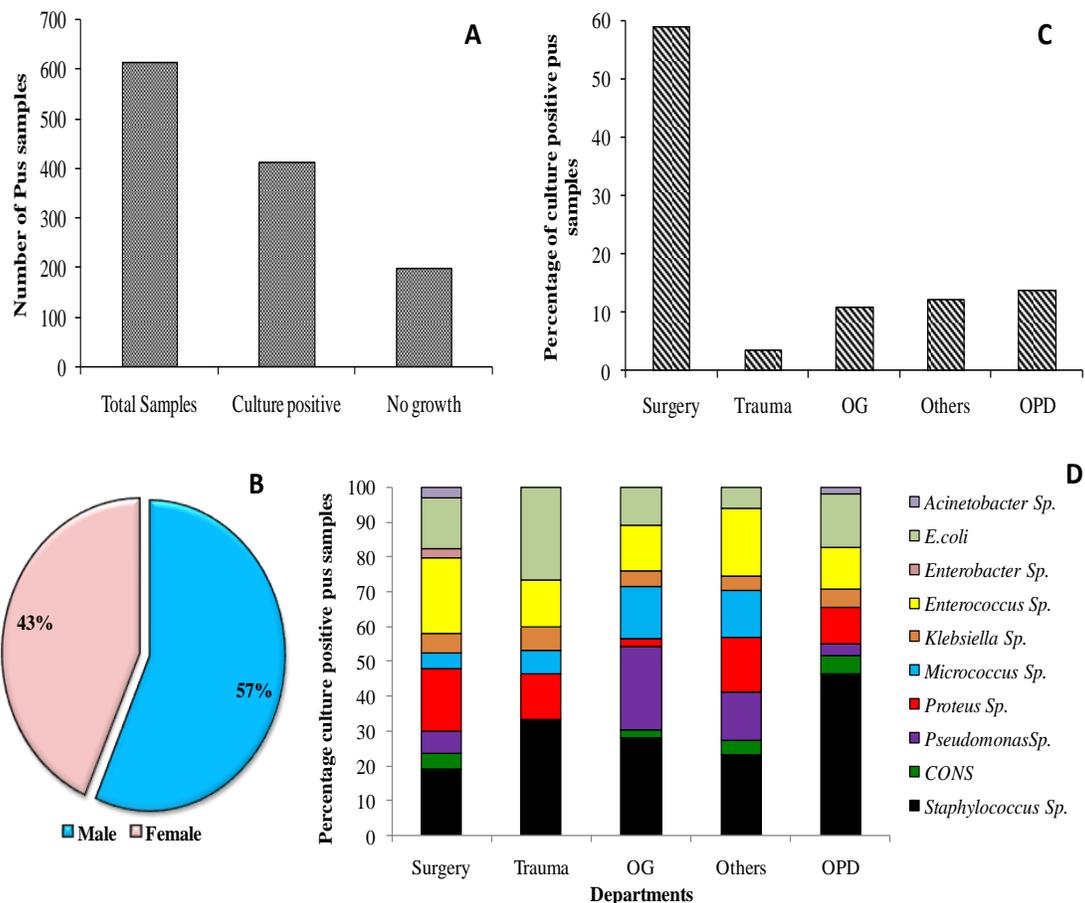


Fig. 1. (A) Distributions of culture positive cases, (B) Gender wise distribution of patients with pathogen positive samples, (C) department wise distribution of pus samples and (D) department wise distribution of bacterial pathogens.

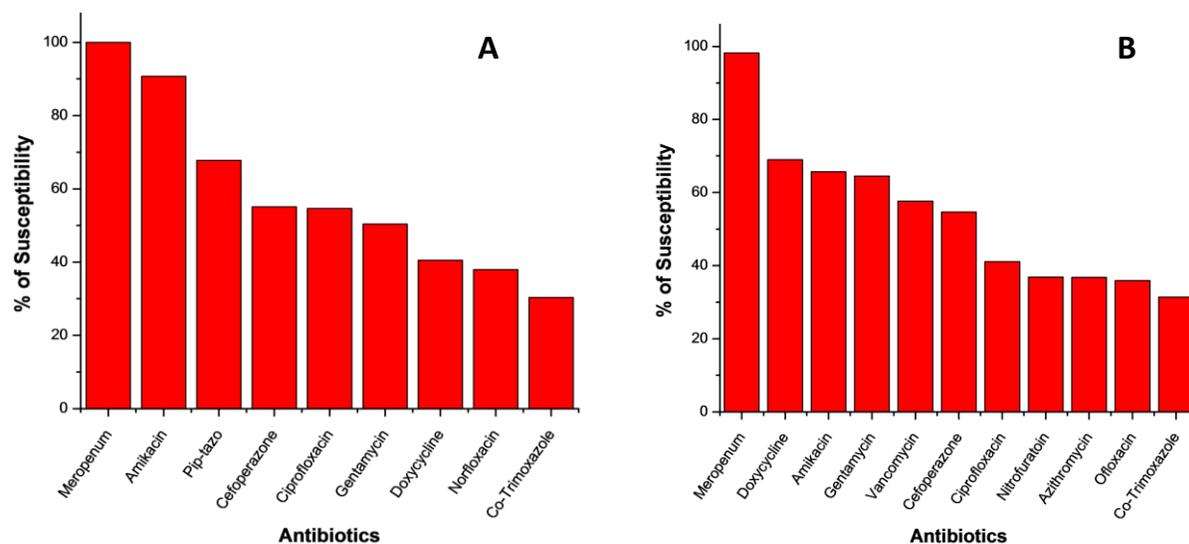
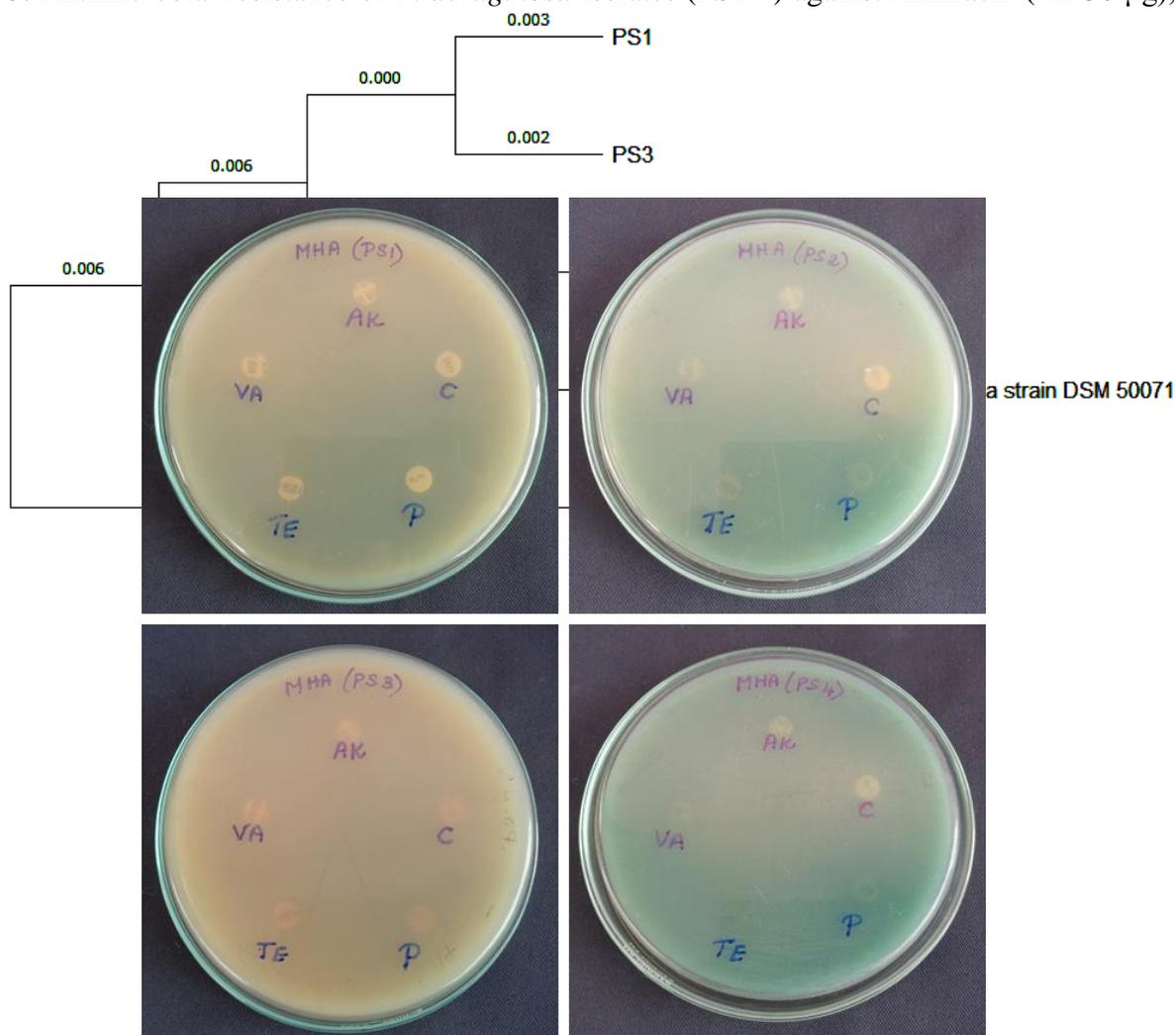


Fig. 2. Antibiotic susceptibility pattern of (A) Gram positive and (B) Gram negative Bacteria isolated from pus samples

Four *Pseudomonas* isolates (PS1-4) were found to be resistant to Amikacin (30 µg), Chloramphenicol (25 µg), Penicillin- G (2U/Disc), Tetracycline (30 µg) and Vancomycin (30 µg) (Fig 3). The four resistant isolates were subjected to 16S rRNA sequencing followed by phylogenetic tree construction. The evolutionary history was deduced by Maximum Likelihood method and Tamura-Nei model (Tamura and Nei 1993). The four isolates were closely similar to the *Pseudomonas aeruginosa*. Around 46 isolates of *Pseudomonas aeruginosa* were isolated mainly from pus, wound swab, sputum and tracheal aspirate with 63.04% *P. aeruginosa* isolates resistant to Ceftazidime, 65.21% to Cefixime, 56.52% to Ceftriaxone and Cefotaxime followed by 56.52% to Piperacillin (Pokharel et al., 2019). Similarly, among 200 *P. aeruginosa* isolates from tertiary care hospital, Faisalabad, majority (n=82) were isolated from pus samples with resistance to β-lactam drugs including carbapenems followed by 95% to levofloxacin and 67% to doxycycline (Qureshi et al., 2018).

Fig. 3. Antimicrobial resistance of *P. aeruginosa* isolates (PS1-4) against Amikacin (Ak-30 µg),



Chloramphenicol (C-25 µg), Penicillin- G (P-2U/Disc), Tetracycline (TE-30 µg) and Vancomycin (VA-30 µg)

Fig. 4. Evolutionary analysis of antibiotic resistant *Pseudomonas* sp. by Maximum Likelihood method

CONCLUSION

A superlative understanding of the causative microbial pathogens present in the wound/pus infections and their sensitivity and resistance profiles to the currently used antibiotics are discussed in the current work. It has been concluded that the pus infections are polymicrobial in nature, and in most cases were associated with *Staphylococcus*, *Klebsiella*, *Escherchia*, *Pseudomonas aeruginosa*, *Enterococcus proteus*, *Micrococcus* etc. Antibiotic resistance is a serious problem our results also displayed high rate of antibiotic resistance in all pathogens isolated. Of all the antibiotics tested, meropenem and amikacin was shown to be the one most likely to be effective in

treating Gram positive and negative bacteria as, in contrast to other antibiotics tested in this study. Four *Pseudomonas* isolates (PS1-4) were found to be resistant to amikacin, chloramphenicol, penicillin- G, tetracycline and vancomycin. The four resistant isolates were confirmed to be *Pseudomonas aeruginosa* as confirmed by 16S rRNA sequencing followed by phylogenetic tree construction. A continuous examination is necessary to examine the susceptibility of the pathogens and to choose correct regimens for prophylaxis and treatment.

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