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In–vitro antibiotic susceptibility pattern for gram positive bacterial pathogens isolated from pus sample in India

Shefali Kansal, Pankaj Kumar Jiloha*

Department of Microbiology, Kurukshetra University, Kurukshetra, Haryana (India)

Abstract: Pus is a whitish yellow or yellowish substance which is made of dead leukocytes and dead bacteria. Pus infection, due to various bacteria has a significant impact on public health in the region so early diagnosis and appropriate treatment remain important. The present study was carried out for a period of 3 months (February to April 2014) in Microbiology Laboratory of General Hospital Panchkula (Haryana), to isolate, identify and screen various bacterial pathogens present in pus sample and to determine their antibiotic sensitivity and resistance pattern against the commonly used standard antibiotic. A total of 63 pus samples were collected and analyzed. Out of total 63 samples, 42 bacterial isolates of 6 species were isolated which included 2 species of gram positive bacteria and 4 species of gram negative. 21-30 age groups were found to be the most vulnerable age group in both male and female. *In-vitro* study determine that the most common gram +ve bacterial pathogens isolated and identified from pus sample were *Staphylococcus aureus* and *S. epidermidis,* both are facultative anaerobe but *S. aureus* was found to be most predominant followed by *S. epidermidis*. During antibiotic susceptibility pattern, the most sensitive antibiotic was Vancomycin (100%) while the least effective antibiotic was Amoxicillin (35%) followed by Penicillin (36%) respectively. Our study reveals that bacterial pathogens showed resistance to most of the antibiotics. Penicillin, Ampicillin, Ciprofloxacin, Ofloxacin, Gentamycin antibiotic were showed high resistance to prevent from gram positive bacteria. So, it may be concluded that the high prevalence of *S. aureus* infection treated with the use of appropriate antibiotic i.e. the use of Vancomycin.

Keywords: Pus infection, bacterial pathogens, standard antibiotics, *Staphylococcus*

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Infection may be defined as the successful entry, lodgement and multiplication of disease causing microorganisms such as bacteria, fungus, viruses and parasites into the host and producing harmful effects to the host (Bansal's New Medical Dictionary, 2002). The most frequent entry of disease causing bacteria into the body are the sites where mucous membrane meets with the skin such as respiratory tract (upper and lower airways) and gastro-intestinal (primarily mouth) tract. Abnormal areas of mucous membrane and skin (e.g. cuts, burns, and other injuries) are also important sites for entry of microorganisms (Brooks et al. 2007). The exposure of subcutaneous tissue following loss of skin integrity provides a moist warm and nutritive environment for growth of microorganisms and its colonization. However, the abundance and diversity of pathogenic microorganisms in any wound infection, influenced by many factors such as wound type, depth, location, quality, the level of tissue perfusion etc. (Bowler et al. 2001). Pus infection is a very common infection not only in India but all around the world and is causing a great fear both in the developed and the developing countries. In pus infection microorganisms invade the host protective layer i.e. the skin and thus disturb the function of the layer (Verma, 2012). Various types of wound infection such as surgical wound infection, acute soft tissue infection, bite wound infection; burn

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Corresponding Author:
Jiloha PK, (✉) Department of Microbiology, Kurukshetra University, Kurukshetra, Haryana (India)
email: pkpkmicro632@gmail.com

wound infection, pyogenic wound infection, leg and decubitus (pressure) ulcer infections caused by a no. of bacteria. Major skin infections like impetigo are caused by *S. pyogenes* or *S. aureus* and both gains minor trauma to skin is a part of everyday life. Boils and furuncles are the most frequent lesions caused by *S. aureus* (Betty et al. 2002). Surgical wound and skin infections caused one third of the nosocomial infections among various surgical patients. They are an important cause of morbidity and 70-80% mortality (Zafar et al. 2007). For control of wound infections in hospitals there is an urgent need of frequent and timely conversation between the microbiologist and wound care practitioners (Giacometti et al. 2000). The presence of necrotic (dead) tissue, blood clots or other foreign material, such as dirt and mud, also increases infection risk, as all of these provides a habitat for microbial multiplication (Patel, 2007).

Today a lot of difficulties occur in treating various diseases because of antibiotic resistance development by pathogenic microorganisms. Resistance by disease causing organisms to antimicrobial drugs is a common problem to all over the world. Infections caused by resistant microorganisms fail to respond to conventional treatment hence causing illness and hospitalization and greater risk of death (Ratheesh et al. 2015).

For example methicillin resistant Staphylococcus aureus (MRSA), Serratia mercescens vancomycin resistance enterococci (VRE) and extended spectrum beta- lactamase (ESBL) producing enterococci are the source of nosocomial infection (Patil et al. 2013; Samson and Anthony, 2013). So, to protect from disease-causing organisms a best option is antibiotic therapy and antibiotic susceptibility test. The goal of antibiotic susceptibility testing is to determine failure or success of antibiotic therapy and to control the different antibiotic resistant pathogenic microorganisms (Verma, 2012).

The aim of present study was to isolate and identify the various pathogens present in pus samples with relation to age and gender and their antibiotic susceptibility pattern to various antibiotics.

MATERIALS AND METHODS

Study area

This study was carried out in the General Hospital, Panchkula and in the Dept. of Microbiology, Kurukshetra University, Kurukshetra (Haryana) India.

Study design and Sample collection

This paper is a part of study that was performed during a 3 month period (from Feb to April 2014) in General Hospital, Panchkula. According to standard techniques, 63 specimens of pus (surgical, acute soft tissue, bite, burn, diabetic foot ulcer, nosocomial) were collected from different patients. For the study, pus samples were collected on a sterile cotton swab or aspirated in syringe and labeled with date, time, patient's name, age and sex. For laboratory investigation, two pus swabs were collected; one for the direct smear preparation and the other one for culture (Koneman et al. 2005).

Macroscopic observation

The physical characteristics of the pus samples were noted. The pus samples were examined for its odour, colour, presence of tissue and blood.

Microscopic observation

The specimen was evenly spread on clean, sterilized glass slide to prepare smear. The smear was air dried, heat fixed and stained by Gram staining, observed under microscope and examined for the type and morphology of bacteria (Koneman et al. 2005).

Isolation and identification of samples

Specimens were cultured on Nutrient agar (NA), Mac-Conkey agar (MA), Blood agar (BA) and incubated at 37°C under aerobic condition in an incubator. Culture was performed with the help of cotton wool swab by dipping it into the inoculum and streaking the swab all over the surface of medium, rotating the plate through an angle of 60^0 after each application following the methodology described by National Committee for Clinical Laboratory Sciences. Finally, pass the swab into the edge of agar surface. Identification of isolates was done on the basis of colony morphology, gram staining, motility, oxidase test, use of selective media and by various biochemical tests (NCCLS, 1993; Aneja, 2003).

Antibiotic sensitivity test for isolated pathogens

Antibiotic discs used

Commercially available antibiotic discs for bacteria in market such as Amoxicillin (10 mcg), Ciprofloxacin (5mcg), Cefotaxime (30mcg), Gentamicin (10mcg), Erythromycin (15mcg), Ofloxacin (5mcg), Penicillin (10 units), Vancomycin (30mcg), Clindamycin (2mcg) and Ampicillin (10mcg) were used for antibiotic susceptibility test (NCCLS, 1993).

Preparation of bacterial strain inoculum and procedure

The inoculums of isolated bacterial strains were prepared in 5 ml nutrient broth with 3 to 5 colonies of each bacterial strain. The inoculums were incubated at 37°C for 24 hrs. A sterile cotton swab was dipped into the cell suspension of the respective isolate inoculated on the entire nutrient agar surface of each plate first in a horizontal direction and then in a vertical direction to ensure even distribution of the organisms. Antibiotic discs are placed after 5 minutes to allow the agar surface to dry. The inoculated plates were incubated at 37°C for 24-48 hr in an inverted position and the zone of inhibition was recorded. After overnight incubation, the diameter of each zone (including the diameter of disc) were measured and recorded in mm (NCCLS, 1993; 2000).

RESULTS AND DISCUSSION

A study was performed in the Microbiology laboratory of General Hospital Panchkula (Haryana), to isolate, identify and screen various bacterial pathogens present

in pus sample and to determine their antibiotic sensitivity and resistance pattern against the commonly used standard antibiotic, a total of 63 pus samples were collected and analyzed. For isolation of the pathogens, samples were directly inoculated to Nutrient agar (NA), Mac-Conkey agar (MA) and Blood agar (BA). Out of 63 samples, 42 bacterial isolates of 6 species were isolated which included 2 species of Gram positive bacteria and 4 species of Gram negative. Age groups (21-30) were found to be the most affected age group and we observed that both female and male were suffered from infection (Table 1). Identification of various isolates was done on the basis of morphological characteristics, gram staining, oxidase test and various biochemical tests performed in the laboratory. The most common bacterial pathogens isolated and identified from pus sample were *Staphylococcus aureus* and *S. epidermidis* but *S. aureus* was found to be most predominant followed by *S. epidermidis*. During antibiotic susceptibility pattern, Vancomycin (100%) was found to be the most sensitive antibiotic followed by Cefotaxime (82.66%) and Amoxycillin (72%) while Ampicillin (48%) and Penicillin (28%) being the least sensitive antibiotic respectively (Fig. 1 & 2). Our results revealed that isolated pathogens showed resistance to most of the antibiotics. Penicillin, Ampicillin, Ciprofloxacin, Ofloxacin and Gentamycin showed high resistance to prevent from gram positive bacteria. The diameter of each zone (including the diameter of disc) was measured and recorded in mm with the help of Hi Zone Scale or calibrated ruler (Table 2).

In our study, *Staphylococcus aureus* was the most predominant bacteria followed by *S. epidermidis* and previously most published reports concluded that *Staphylococcus* has been the predominant species isolated from various cases of pus, wounds and swab samples as earlier studies have indicated (Bell and Jounidge, 2002; Sharma et al. 2006; Verma, 2012) which supported our research. During a current study done by Ratheesh et al (2015), in pus culture the most common organism isolated was *S. aureus* (64.4%) followed by *Pseudomonas* (22.03%) and *Klebsiella* (13.6%). The *S. aureus* showed 79% sensitivity to vancomycin, 70.8% to linezolid, 42.7% to cephalothin, 43.7% to erythromycin, 2.6% to ofloxacin and 2% to ciprofloxacin and was totally resistant to ampicillin and penicillin. Similar results were published by Manikandan and Prabhakaran

Table 1. Age and gender wise distribution of patient with positive cases

Age group (years)	Gender				M+F	M+F (Positive cases)	M+F (%)	M+F (Positive cases)%
	Male (M)		Female (F)					
	No.	+ve Case	No.	+ve Case				
Below 11	2	2	1	1	3	3	4.76	0.04
11-20	5	4	4	3	9	7	14.28	11.11
21-30	12	6	9	6	21	12	33.33	19.04
31-40	8	4	6	5	14	9	22.22	14.28
41-50	5	4	4	2	9	6	14.28	9.52
51-60	4	3	3	2	7	5	11.11	7.93
Total	36	23	27	19	63	42	100	66.66

Table 2. Diameter of Drug sensitivity

Antibiotic	Disc content	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Ofloxacin	5mcg	16	13-15	12
Penicillin	10 units	29	-	28
Amoxicillin	10 mcg	20	-	19
Erythromycin	15mcg	23	14-22	13
Cefotaxime	30mcg	23	15-22	14
Ciprofloxacin	5mcg	21	16-20	15
Gentamicin	10 mcg	15	13-14	12
Ampicillin	10mcg	17	14-16	13
Clindamycin	2 mcg	21	15-20	14
Vancomycin	30 mcg	17	15 -16	14

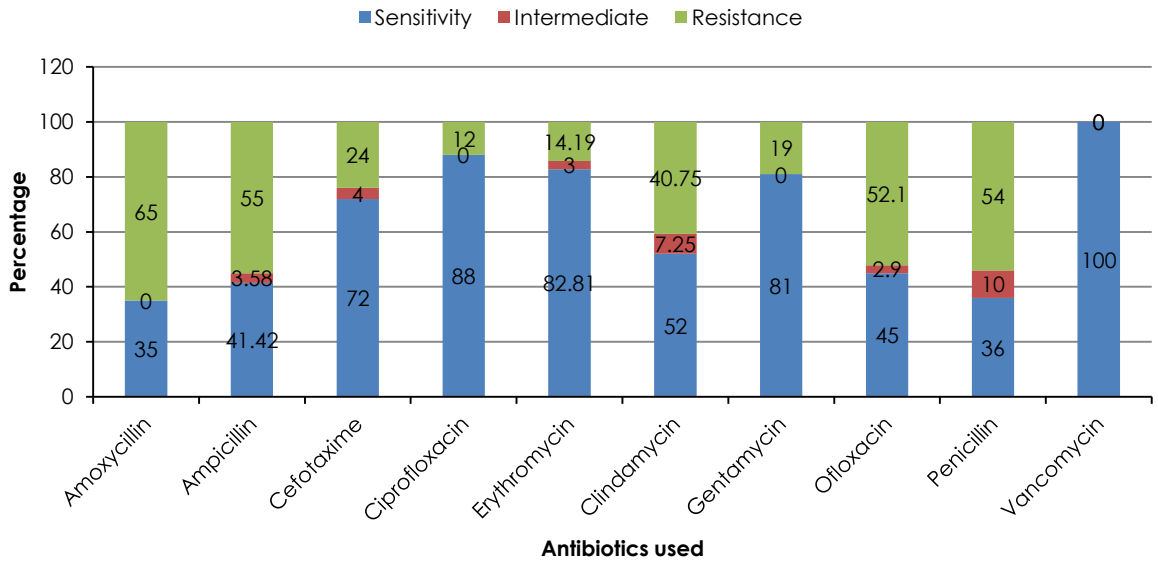

Figure 1. Antibiotic susceptibility pattern of *Staphylococcus aureus*

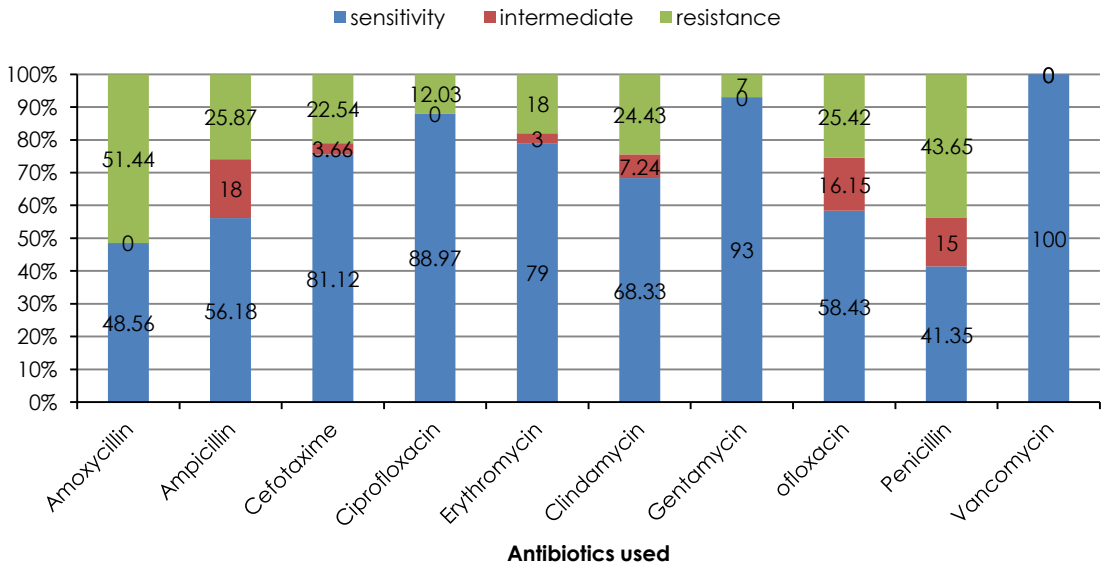

Figure 2. Antibiotic susceptibility pattern for isolates of *Staphylococcus epidermidis*

(2015) i.e. the gram positive isolates were found to be sensitivity to amikacin and gentamicin (100%) followed by ofloxacin (92.4%), vancomycin (90.1%), ciprofloxacin (79.3%), erythromycin (77.3%), amoxyclavate (74.6%), clindamycin (59.7%), methicillin (54.1%) and ampicillin (48.6%), which supported our research.

Nagaraju and Divakar (2012) reported that gram positive bacteria were most sensitive to Ciprofloxacin (100%) and Chloramphenicol (94%) but our results showed gram positive bacteria was most sensitive to Vancomycin (100%) and Cefotaxime (82.66%). Similarly Patil et al (2013) in Maharashtra (India) reported that the most dangerous bug i.e. Methicillin resistant Staphylococcus aureus (MRSA) was 100% resistant to every antibiotic of Cephalosporin group and observed that sensitivity against β – Lactamase inhibitors (PIT:

100%), Phenicol (C:91%), Quinolones (GAT and MO: 100%), Glycopeptides (VA: 100%) and Aminoglycosides (AK and NET: 82%) which supported our results. Kumar (2013) also reported that S. aureus has showed good sensitivity to glycopeptide antibiotic i.e. Vancomycin and resistant to Amoxicillin, Penicillin-G and Chloramphenicol. So, on the basis of comparison of different results it may be concluded that better control of gram positive infection can be prevent by the use of Vancomycin.

Conclusion

Pyogenic infection has been the major cause of morbidity since a long time. Emerging multidrug resistant (MDR) strains is of major concern to treat these conditions. Even though gram negative bacteria are being increased significantly but still *Staphylococcus aureus* is being continued as a major etiological agent of pyogenic infections. Appropriate and judicious selection of antibiotic by using antibiotic sensitivity data would limit the emerging drug resistant strains in the future to treat these clinical conditions successfully. In conclusion, proper management of pus infection with the use of appropriate antibiotic must be necessary to control life threatening disease and to control incidence of drug resistance development by pathogenic microorganisms.

References

Aneja KR, 2003. Experiments in Microbiology, Plant Pathology and Biotechnology. 4th ed. New Delhi: New Age International Publishers

Bansal's New Medical Dictionary, 2002. 1sted. A.I.T.B.S. Publishers and Distributors (Regd.)

Bell JM, Jounidge JD (2002) Higher prevalence of oxacillin resistant Staphylococcus aureus isolates from hospital patients in Asia-pacific and South Africa: Results from sentry antimicrobial surveillance program. Antimicrobial Agent Chemothe 4:56-62

Betty AF, Daniel FS, Weissfeld AS, Bailey S (2002) Diagnostic Microbiology. 11th ed. Mosby-Year Book. pp. 972-982

Bowler PG, Duerden BI, Armstrong DG (2001) Wound Microbiology and associated approaches to wound management. Clin Microbiol Rev 14(2):244-269

Brooks GF, Butel JS, Morse SA, Jawetz M (2007) Adelberg's Medical Microbiology. 22nd ed. Lange medical books. pp. 161-170

Giacometti A, Cirioni O, Schimizzi AM, Prete Del MS, Barchiesi F, D'Errico MM, Petrelli E, Scalise G (2000) Epidemiology and Microbiology of surgical wound infections. J Clin Microbiol 38(2):918-922

Koneman WK, Allen SD, Janda WM, Schreckenberger PC, Propcop GW, Woodsand GL, Winn WC (2005) Philadelphia Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott-Raven Publisher, 624-662

Kumar R (2013) Antimicrobial sensitivity pattern of Staphylococcus aureus isolated from pus from tertiary care hospital, surendranagar, Gujarat and issues related to the rational selection of antimicrobials. Sch J App Med Sci 1(5):600-605

Manikandan C, Prabhakaran P (2015) Clinical and bacteriological profile of diabetic foot infections in Pattukkottai area hospitals, Tamilnadu, India. Int J Curr Res Aca Rev 3(4) 166-173

Nagaraju VE, Divakar G (2012) Antibiotic susceptibility of bacterial strains isolated from diabetic patients. Int J Adv Pharma Biol and Chem 1(4)

NCCLS (2000) Performance standards for antimicrobial susceptibility testing. 7th ed. Wayne, PA: Approved standard M2-A7. National Committee for Clinical Laboratory Standards

NCCLS (National Committee for Clinical Laboratory Standards) (1993) Dilution antimicrobial susceptibility for bacteria that group aerobically. Approved standards, NCCLS Document, pp: M7-A3

Patel (2007) Understanding wound infection and colonization; wound essentials- Technical guide. (2):132-142

Patil A, Patil K, Pawar P, Maheshwari V (2013) Survey of antibiotic sensitivity in nosocomial infections in North Maharashtra region. J Asso Physi Ind 61:18-20

Ratheesh R, Mohandas B, Sahadevan P, Venugopalan P (2015) "A retrospective analysis on antimicrobial sensitivity pattern in a medical college hospital in Kannur district". J Evol Med Dental Sci 4(37): 6443-6450

Samson OO, Anthony EO (2013) Prevalence of multi-drug resistant Staphylococcus aureus in clinical specimens obtained from patients attending the University of teaching hospital, Benin City, Nigeria. J Natural Sci Res 3(5)154-159

Sharma VK, Khadka PB, Joshi A, Sharma R (2006) Common pathogens isolated in diabetic foot infection in Bir hospital, Kathmandu University. Med J pp: 4295-301

Verma P (2012) Antibiotic sensitivity treatment for gram positive bacteria isolated from pus sample. Bull Environ Pharmacol Life Sci 1(10):03-06

Zafar A, Anwar N, Ejaz H (2007) Bacteriology of infected wounds-A study conducted at children hospital Lahore. Pak J Med Health Sci 1(3):92-95