Study Of Most Common Bacterial Isolates Causing Pyogenic Infections.

Shubhi Taneja[1], Gurinder Singh[2]

Department Of Medical Laboratory Sciences, Lovely Professional University, Punjab (India)-144411
E mail: gurinder.singh@lpu.co.in;

ABSTRACT
Pyogenic infections are the challenges in the hospital patients invite to other nosocomial infections.It is also life threatening and required appropriate antibioticstotreatthesepyogenicbacterialinfections. Multi drug resistance is accountable to make chemotherapy more difficult to manage the bacterial pyogenic infections.
Objective: To determine the antimicrobial susceptibility pattern of bacterial isolates from pyogenic infection.
Methods:A study was conducted on samples collected differentwardsofthehospital.Pussamplewascollectedthroughaspirationandsterileswabs.The clinical specimens were processed immediately after collection to isolate and identification of the pyogenic bacteria Further AST was conducted by automated technique as well as Kirby-Bauer method by following the recommendedprocedures.
Result: Overall 103 specimens were collectedand 44 samples were isolated out of them, showinganisolationrateof42.7%.
Outof44positivesamples19weregrampositive(43.2)and25were gramnegative(56.2%).Themajorbacteriaisolatedfrompuswas Staphylococcusaureus14(32%), followedbyKlebsiellapneumonia6(13%)andEnterobacterspp6(13%),Escherichiacoli5(11%), Pseudomonas aeruginosa 4 (9%), Acinetobacter baumannii 2 (6%), Coagulase negative staphylococci 3 (8%), Enterococcus spp. 2 (4%). The remaining isolates were nonbacterial microbial infections.
Conclusion: Emerging of MRbacterial strainsis the main focusfor thetreatment of pyogenic infections.Major challenges of pyogenic infections are due to Gramnegative bacteria which are followed by Gram positive bacteria such as Staphylococcus aureus. The change in antimicrobial pattern of antibioticsis tested by antibiotic sensitivity testing to treat the challenge in treating these conditions.
Keywords: Pyogenic infections, antibiotic resistance, multi-drug resistance, Gram positive, Gram negative.

1. INTRODUCTION:

Two forms of pyogenic skin infection occur and each infection reveals the various ways skin and deeper tissue respond to two forms of bacterial infection such as*Staphylococcus aureus* and *Streptococcus pyogenes*. *Staphylococcus aureus* is a bacterium normally isolated from infected wounds that is contained as a natural flora in the noses and on the skin of a high proportion of everyday people. Wounds provide very suitable sites with reduced tolerance to bacterial invasion for *Staphylococcal* invasion (Singh et al., 2013). In the ward the organism can also spread from patient to patient during surgical dressing procedures. *Staphylococcus aureus* strains which are immune to antibiotics are not unusual in hospitals.

They are sometimes named ' staphylococci in the hospital'(Trojan et al., 2016). Many of the species usually contained in infected wounds are those normally found in the faeces which include ' coliforms ' (*Escherichia coli* and others), *Proteus, Pseudomonas and Streptococcus faecalis (*Kamatet al., 2017). Such species can be found on the thighs, the knees, and the lower abdomen and sometimes on the head.(Vyas, P., et al 2019) It is therefore not surprising that wounds may become infected with these bacteria, especially those of the lower abdomen (Verma et al., 2012). The emergence of antibiotic resistance and its rapid spread among pathogenic bacterial isolates is seen as a serious challenge to global public health. Multidrug resistant Gram negative and Gram positive have been associated with pus infections in clinical settings due to insufficient use of antibiotics (Rao et al., 2014).

Recent studies found that there is development of resistance in the microbes from routine drugs used against the pyogenic infections with the passage of time (Rao et al., 2014). It is accountable for change the standard protocol of treatment, which develop the need to produce the new drugs by pharmaceutical industries.(Karnwal, A., et al. 2020) This challenge increases the budget cost and side effects in the hospital. It also increases the risk of death due to hospital acquired infections (Victor et al., 2013). Additionally, the level of resistance in the microbes will increase globally but it is raising more in developing countries as compared to developed countries due to unnecessary use of drugs (Roy et al., 2017).

The most common bacterial isolate causing pyogenic infection was*Staphylococcus aureus*(49.28 %). Now researchers are paying attention on raising the level of MDR in pyogenic pathogenic microbes because it would be life threatening in future globally.(Singh, S.,et al 2020, Singh, S., et al 2020)The most vigorous organism creating a challenge to treat the pyogenic infections is *Staphylococcus aureus*(Rijalet al., 2017.,Anguzu et al., 2997). It was observed that *Staphylococcus aureus* is highly resistant to penicillin as compared to ampicillin (penicillin 84.5 % and ampicillin 63.6 %). Macrolides such as erythromycin displayed a sensitivity of about 58.3 % (KC et al., 2013, Kaur, P.,et al 2020).Staphylococcus is very challenging because it istremendously resistant to first and second line of treatment (Khanam et al., 2018., Nwachukwu et al., 2009).

2. METHODS ANDMATERIALS

Studydesign:

A four-month research was performed in the Microbiology Department of a multi-speciality hospital in Delhi from January 2019 to April 2019. A total of 103 pus samples were taken from skin.

Samplecollection:

Specimens were obtained before the use of any drug and dressing of wound. Samples were taken aseptically in a sterile leak proof bottle, syringe or swab and sent to the laboratory for bacterial isolation and further antimicrobial sensitivity testing. Swabs are mostly not preferred due to less volume of sample and chances of contamination from normal microflora of body

Processing ofsample:

*Physicalexamination***:**

Appearance of the specimen was described i.e. the quantity, color, presence or absence of sulphur granules.

Microscopic Examination:

*For pus***:** If only one aerobic swab was provided, the culture media was inoculated to make smears for Gram stain and AFB stain, before using the swab. Where two swabs (one anaerobic and two aerobic) were obtained for cultivation, the second swab was used to make Gram smears (Cheesbrough et al., 2006).

*For aspirates***:** One drop of pus was mounted on a clean microscope slide using a sterile pipette and it was spread using a sterile loop to give Gram's staining a thin smear.

Isolation:

Microorganism isolation was done by extending the sample over 3 agar plates i.e. Blood agar, MacConkey agar, and mannitol salt agar. Plates were aerobically incubated for 24 hours at 37C. Growth (if any) was recognizedby use of cultural characteristics, Gram differential stain and morphological aspects of the colonies were identified and biochemical analysis was pursued with the automated Vitek 2 system. The pattern of antimicrobial susceptibility for each previously identified bacterial species was determined using test cards Vitek 2.

Those practices followed instructions from the supplier.

Antibiotic susceptibilitytesting:

The ASTwasdonebyVitek2Compactsystem.Gram- positiveandgram-negativebacteriawerefiguredoutonthebasisofculturalcharacteristicsandgram- staining. In a polystyrene tube 3 ml of Vitek saline was taken. With the help of a sterile loop the separated individual colonies were inoculated to reach to set a density of .49 to .61. Density was checked by DensiChek. In another polystyrene tube with 3 ml Vitek saline, a dilution is made by transferring 145 □l (for gram-negative bacteria) and 280 □l (for gram-positive bacteria) of the inoculated tube to the later tube. The identification cards were placed in the inoculated tube and the AST cards were placed in dilution tubes. For gram negative, oxidase negative bacteria GN card was implemented for recognition and AST 280 card for AST and for gram negative, oxidase positive AST 281 and AST 235 cards were used for antibiotic susceptibility testing instead of 280. For gram positive bacteria GP card were used for identification and AST P628, AST STO1 card for antibiotic susceptibilitytesting(Bauer et al., 1966).

3. RESULT:

Inthisstudy,103samplesaspiratesandswabs weretested forcultureandsensitivityoutofwhich 44werefoundpositivegivinganisolaterateof42.7%.Themicrobiologicalanalysisrevealedthat *Staphylococcus aureus* was the most prominent bacterial agent causing pyogenic infections. *Staphylococcusaureus*istheleadingetiologicalagentofinfectioninthegivenhealthcaresetting. Outof44positivesamples19weregrampositive(43.2)and25weregramnegative(56.2%).The largest isolated microbes from pus was *Staphylococcus aureus 14 (32%),* followed by *Klebsiella pneumonia 6 (13%)* and *Enterobacter spp6 (13%), Escherichia coli 5 (11%), Pseudomonas aeruginosa 4 (9%), Acinetobacter baumanni2 (6%), Coagulase negativestaphylococci 3 (8%), Enterococcus spp. 2 (4%).*

Staphylococcus aureus showed resistance to Benzylpenicillin, Levofloxacin, Ciprofloxacin and Erythromycin, an intermediate susceptibility was seen for Oxacillin and remaining antibiotics i.e. Nitrofurantoin, Tigecycline, Gentamicin High Level, Clindamycin, Linezolid, Daptomycin, Teicoplanin, Vancomycin, Tetracycline and Rifampicin were found susceptible. *Enterococcus spp*were resistant to Benzylpenicillin, Levofloxacin Ciprofloxacin, Erythromycin, Gentamicin High Level and Tetracycline.
Klebsiella pneumoniae was found resistant to Ampicillin, Amoxicillin/Clavullanic acid, Ticarcillin,Piperacillin/Tazobactam, Cefuroxime, Cefuroxime Axetil, Cefoperazone/Sulbactam, Cefepime, Cefalotin, Cefoxitin, Cefixime, Ceftazidime, Ceftriaxone, Ertapenem, Amikacin, Gentamicin, Nalidixic acid, Ciprofloxacin, Ofloxaxin, Nitrofurantoin, Trimethoprim/Sulfamethoxazole, Meropenem, Imipenem, Tigecycline, Tobramycin, Ampicillin/Sulbactam, Netilmicin, Ticarcillin/Clavulanicacid.
*Escherichiacoli*wasfoundsensitivetoAmoxicillin/Clavullanicacid,Piperacillin/Tazobactam, Cefoperazone/Sulbactam, Cefepime, Cefoxitin, Ceftazidime, Ertapenem, Amikacin, Gentamicin, Nitrofurantoin, Meropenem, Imipenem, Tigecycline, Ampicillin/Sulbactam, Netilmicin.
Enterobacter spp. was found sensitive to Trimethoprim/Sulfamethoxazole, Ampicillin/Sulbactam.

Pseudomonas aeruginosa was susceptible to Piperacillin/Tazobactam, Cefoperazone/Sulbactam, Cefepime, Ceftazidime, Ciprofloxacin, Meropenem, Imipenem, Tigecycline, Ticarcillin/Clavulanic acid, Levofloxacin, Doripenem, Aztreonam.
*Acinetobacter baumannii*was only sensitive to Tigecycline.

ANTIBIOTICS	**BACTERIAL STRAIN SUSCEPTIBILITY (%)**	
	Staphylococcus aureus	***Enterococcus spp.***
NITROFURANTOIN	100%	75%
TIGECYCLINE	100%	100%
BENZYLPENICLLIN	0%	25%
OXACILLIN	52.6%	-

GENTAMICIN HIGH LEVEL	80%	25%
CIPROFLOXACIN	0%	0%
LEVOFLOXACIN	0%	25%
ERYTHROMYCIN	26.3%	0%
CLINDAMYCIN	80%	-
LINEZOLID	100%	-
DAPTOMYCIN	100%	-
TEICOPLANIN	100%	62.5%
VANCOMYCIN	100%	50%
TETRACYCLINE	80%	0%
RIFAMPICIN	89.4%	-

Table 1: Percentage susceptibility of Staphylococcus aureus and Enterococcus spp.

ANTIBIOTICS	**BACTERIAL STRAIN SUSCEPTIBILITY (%)**	
	Escherichia coli	***Klebsiella pneumonia***
AMPICILLIN	20.8%	0%
AMOXICILLIN/CLAVULANIC ACID	65.6%	25%
TICARCILLIN	26.6%	0%
PIPERACILLIN/TAZOBACTAM	92.5%	25%
CEFUROXIME	32.4%	5%
CEFUROXIME AXETIL	32.4%	5%
CEFOPERAZONE/SULBACTAM	90%	15%
CEFEPIME	83.3%	15%
CEFALOTIN	45.9%	0%
CEFOXITIN	86..4%	40%
CEFIXIME	59.4%	0%
CEFTAZIDIME	86.4%	0%
CEFTRIAXONE	41.7%	0%
ERTAPENEM	100%	5%
AMIKACIN	88%	45%
GENTAMICIN	76%	25%
NALIDIXIC ACID	5%	10%
CIPROFLOXACIN	29.8%	20%

OFLOXAXIN	51.6%	38%
NITROFURANTOIN	100%	25%
TRIMETHOPRIM/SULFAMETHOXA	44.7%	15%
ZOLE MEROPENEM	95.3%	20%
IMIPENEM TIGECYCLINE	81.3%	40%
TOBRAMYCIN	100%	- 33.3%
AMPICILLIN/SULBACTAM	26.4%	22.2%
NETILMICIN	58.2%	33.3%
TICARCILLIN/CLAVULANIC ACID	64.1%	0%

Table2.PercentageantibioticsusceptibilityofEscherichiacoliandKlebsiellapneumonia.

ANTIBIOTICS	**BACTERIAL STRAIN SUSCEPTIBILITY (%)**
	Acinetobacter baumannii
PIPERACILLIN/TAZOBACTAM	0%
CEFOPERAZONE/SULBACTAM	16.6%
CEFEPIME	0%
CEFTRIAXONE	0%
AMIKACIN	33.3%
GENTAMICIN	16.6%
CIPROFLOXACIN	16.6%
TRIMETHOPRIM/SULFAMETHOXAZOLE	0%
MEROPENEM	0%
IMIPENEM	0%
TIGECYCLINE	100%

Table 3: Percentage antibiotic susceptibility of Acinetobacter baumanni.

ANTIBIOTICS	**BACTERIAL STRAIN SUSCEPTIBILITY (%)**
	Pseudomonas aeruginosa
PIPERACILLIN/TAZOBACTAM	100%
CEFOPERAZONE/SULBACTAM	80%

CEFEPIME	80%
CEFTAZIDIME	80%
MEROPENEM	80%
IMIPENEM	80%
TIGECYCLINE	80%
TICARCILLIN/CLAVULANIC ACID	60%
CIPROFLOXACIN	60%
LEVOFLOXACIN	60%
DORIPENEM	80%
AZTREONAM	60%

Table 4: Percentage antibiotic susceptibility of Pseudomonas aeruginosa.

ANTIBIOTICS	**BACTERIALSTRAIN SUSCEPTIBILITY (%)**
	Enterobacter spp.
AMOXICILLIN/CLAVULANIC ACID	0%
PIPERACILLIN/TAZOBACTAM	25%
CEFUROXIME	0%
CEFUROXIME AXETIL	0%
CEFTRIAXONE	25%
CEFOPERAZONE/SULBACTAM	25%
CEFEPIME	25%
IMIPENEM	50%
MEROPENEM	50%
AMIKACIN	50%
GENTAMICIN	50%
NALIDIXIC ACID	25%
CIPROFLOXACIN	25%
NITROFURANTOIN	0%
TRIMETHOPRIM/SULFAMETHOXAZOLE	75%
TOBRAMYCIN	33.3%
AMPICILLIN/SULBACTAM	66.6%
NETILMICIN	33.3%
TICARCILLIN/CLAVULANIC ACID	33.3%

Table 5: Percentage antibiotic susceptibility of Enterobacter spp.

4. DISCUSSION:

In this study it was found out that *Staphylococcus aureus* causes most number of pyogenic infectionmakingittheetiologicalagentofthecause.Staphylococcus aureus is the most common organism associated with surgical wound infections, according to the Center for Disease Control and Prevention (CDC), which is in line with our study results. .*Staphylococcus aureus* is most prevalent infection maybe because it is an endogenous source of infection. Since *Staphylococcus aureus* is the normal flora of nostrils, it can be carried on to the surgicalsite.

Staphylococcus aureus was found susceptible to Nitrofurantoin (100%), Tigecycline (100%), Gentamicin high level (80%), Clindamycin (80%), Linezolid (100%), Daptomycin (100%), Teicoplanin (100%), Vancomycin (100%), Tetracycline (80%) and Rifampicin (89.4%). *Staphylococcusaureus*wasfoundresistanttothefirstlineofantibioticsi.e.Benzylpenicillin(0%), Ciprofloxacin (0%), Levofloxacin (0%) and Erthyromycin (26.3%). An intermediate sensitivity was seen for Oxacillin(52.6%).

Pyogenicinfectionsduetocoliformsandothergram-negativebacteriai.e.*Klebsiellapneumoniae*, *Pseudomonasaeruginosa*,*Enterobacterspp.*,*Acinetobacterbaumannii*areonrise.Alsothestudy reveals multidrug-resistance for several microorganisms. Antibiotic resistance among pyogenic pathogens has been growing slowly, so it is essential to know the trend and antimicrobial susceptibility to choose the right treatment regimen. The research demonstrates high prevalence of antibiotic-resistant bacteria in the pus samples. The antibiotic resistant pattern canbevariablefordifferentlocationsMulti-drug resistance can occur due to patient neglect, incomplete treatment schedules, misuse of antibiotics, self-prescription, misprescription, lack of regional antibiogram data, and limited awareness among clinicians about multi-drug-resistant isolates and antimicrobial resistance. An improved awareness of antimicrobial susceptibility profiles of clinical isolates will assist in the design of the most effective dose-regime and treatment for multiple pyogenic infections and will also help to curb the alarmingly increasing drug resistance issue. One of the most severe and serious complications of the hospital-acquired infections is pyogenic infection. This can get bigger the length of hospitalstay.

5. CONCLUSION:

Emerging of multidrug resistant bacterial strainsis the major concerntotreatthe pyogenic infections. Major challenge is due to Gramnegativebacteriawhich are followed by Gram positive bacteria such as Staphylococcus *aureus*. The change in antimicrobial pattern of antibiotics is tested by antibiotic sensitivity testing to treat the challenge in treating these conditions. Drugresistance has been increasing alarmingly in pyogenicbacteria in developing countries. The MDR is growing due to miss use of antibiotics across the world, mostly in developing countries. This issue can lead to complications in treatment, increase the stay and costs in the hospital. The routine antimicrobial sensitivity testing of patients suffering with pyogenic infections can guide to determine the susceptibility pattern and help in treatment protocols. This study was conducted to isolate the bacteria from the clinical from the patients having pyogenic infection and detect the drug of choice against several available antibiotics. It is suggested that stringent health policies should be executedtocontroltheconsumptionandprescription of antibiotics. There is need torestricttheunverifiedantibioticuseas well as constant checking and reporting of antibiotic resistance.

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