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Original Article

Determining Of Susceptibility & Resistant Pattern Of Bacteria Isolated From Pus Of Various Clinical Specimens

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Abstract:

Background: Antibiotic resistance among pyogenic bacteria has been gradually increasing.Using antibiotics without proper determination of susceptibility and resistant pattern may lead to antibiotic abuse and ultimately antibiotic resistance. It is important to have knowledge about susceptibility and resistant pattern of organisms isolated from pus to choose the correct treatment regimen.

Objective: To determine the antibiotic susceptibility & resistant pattern of pyogenic bacteria isolated from pyogenic infection.

Methods: A cross sectional study was carried out at the Department of Microbiology, Dhaka National Medical College & Hospital from January 2021 to July 2021. A total 200 samples were taken from various clinical specimen containing pus. Samples from cases of Urethritis, Cervicitis, Meningitis, UTI or other Sexually transmitted infection were excluded from this study. Purulent conjunctival swab, ear swab, pus from wound,(surgical and accidental) abscess, were firstly tested by gram staining. Then cultured on Mac Conkey agar media & Blood agar media as well as Chocolate agar media if necessary. Then media were incubated at 37°c for 24-48 hours. Colonies grown on to media were further tested for coagulase test ,biochemical reaction,(oxidase test, and inoculated into soft agar media as TSI,) At last susceptibility and resistant pattern were tested on to Muller-Hinton agar media.

Results: A total 200 samples were taken from various clinical specimen containing pus. Staphylococcus aureus is the predominant among gram positive organisms. Among gram negative bacteria, Klebsiella pneumonia was the predominant organism. Staphylococcus aureus exhibit sensitivity to Vancomycin, Linezolid, Imipenem, Amikacin, Fusidic acid & Amoxicillin -Clavulinic acid combination. Staphylococcus aureus showed highest sensitivity to Vancomycin & exhibit maximum resistant to Cefixime. Pseudomonas showed a very good sensitivity to Amikacin, Imipenem, Tazobactam & Gentamycin but resistant to Ceftazidime and Co-trimoxazole. Pseudomonas is highly sensitive to Amikacin & highly resistant to Ceftazidime. Antimicrobial susceptibility of E.coli showed a very good sensitivity to Imipenem, Amikacin, Amoxiclav, Ceftriaxone & Ciprofloxacin but resistant to Cefixime, Cephradine, Ceftazidime & Cefuroxime . E.coli exhibit maximum sensitivity to Imipenem followed by Amikacin & showed maximum resistant to Cefixime.

Conclusion: This study showed that most common Pyogenic bacteria isolated from various clinical specimen are Staphylococcus aureus, Klebsiella spp, Pseudomonas spp, E. coli & Streptococcus pyogenes. Different bacteria differ in response to different antibiotic therapy. Based on this study it may be concluded that testing of sensitivity and resistant before starting antibiotic treatment is very important to prevent emergence of drug resistance bacterial strain.

Key words: Pus, Pyogenic bacteria, Susceptibility, Resistant pattern.

Introduction

Pyogenic infection refers to bacterial infection that leads to the production of pus. Pyogenic infection is characterized by several local inflammation, usually with pus formation, generally caused by the pyogenic bacteria, which can produce the accumulation of dead leukocytes and infectious agent commonly known as pus.[1]

Body's defense mechanism recruits immune cells into the infection site to fight against bacteria. Accumulation of these cells produces pus, causing pyogenic infection which actually delays the wound healing and may cause complication like wound dehiscence or wound breakdown.[2]

Pus consists of a thin, protein-rich fluid historically known as liquor puris[3] and dead leukocytes from the body's immune response (mostly neutrophils).[4] During infection, macrophages release cytokines, which trigger neutrophils to seek the site of infection by chemotaxis. There, the neutrophils release granules, which destroy the bacteria. The bacteria resist the immune response by releasing toxins called leukocidins. As the neutrophils die off from toxins and old age, they are destroyed by macrophages, forming the viscous pus. Bacteria that cause pus are called pyogenic.[5]

Pyogenic bacteria like Staphylococcus aureus and Streptococcus pyogenes account for greater than 80% of Pyogenic infection of skin and soft tissue.[6] Other gram negative Pyogenic bacteria include E coli, Klebsiella, pseudomonas, proteus spp.[7] Antibiotics to treat these pyogenic bacterial infections are routinely prescribed. Ciprofloxacin, Ceftriaxone, cloxacillin, imipenem, amikacin, Amoxicillin-Clavulinic acid combination are commonly used antibiotics to treat themComplications arising from pyogenic infection of skin & soft tissue by Staphylococcus aureus and Pseudomonous are a major clinical problem owing to wide spread emergence of antibiotic resistance bacterial strain.[8]

Drug resistance of pyogenic bacteria has been found to increase along with the frequency. This resistance can increase complications and costs associated with procedure and treatment. Routine isolation, identification and susceptibility testing of bacteria present several difficulties leading to defects in the determination of local susceptibility patterns which will guide empirical treatment protocol. This study was carried out to determine the antibiotic susceptibility & resistant pattern of pyogenic bacteria isolated from pyogenic infection.

Materials & Methods

A cross sectional study was carried out at the Department of Microbiology, Dhaka National Medical College & Hospital from January 2021 to July 2021. A total 200 samples were taken from various clinical specimen containing pus. Samples from cases of Urethritis, Cervicitis, Meningitis, UTI or other Sexually transmitted infection were excluded from this study. Purulent conjunctival swab, ear swab, pus from wound,(surgical and accidental) abscess, were firstly tested by gram staining. Then cultured onto MacConkey agar media & Blood agar media as well as Chocolate agar media if necessary. Then media were inoculated at 37°c for 24-48 hours. Colonies grown on to media were further tested for coagulase test ,biochemical reaction,(oxidase test and incubated into soft agar media, TSI,). Antibiotic disks containing Amoxycillin, Amoxiclav, Cephradine, Ceftriaxone, Gentamycin, Doxycycline, Ciprofloxacin, Cefuroxime, Amikacin, Imipenem, Cloxacillin, Vancomycin, Cefixime, Linezolid, Fusidic acid, Azithromycin, Erythromycin, Colistin, Tazobactam were used. At last susceptibility and resistant pattern were tested by disk diffusion method on Muller-Hinton agar media and minimum inhibitory concentration, zone of inhibition were obtained.

Results

A total 200 samples were taken from various clinical specimen containing pus. Among these samples, positive culture was found in 163 (81.5%) samples & negative culture was found in 37 (18.5%) samples [Table I]. Among the positive culture, gram positive bacteria was found in 75 (46.01%) isolates & gram negative bacteria was found in 88 (53.88%) isolates [Table II]. In the gram positive growth, Staphylococcus aureus was found in 70 (93.33%) isolates & Streptococcus pyogenes was found in 5 (6.67%) isolates [Table III]. In the gram negative growth, Klebsiella was found in 45 (51.14%) isolates & Pseudomonas was found in 26 (29.54%) isolates & E. coli was found in 17 (19.32%) [Table IV]. Antimicrobial susceptibility pattern of Staphylococcus aureus was evaluated. Staphylococcus aureus exhibit sensitivity to Vancomycin, Linezolid, Imipenem, Amikacin, Fusidic acid & Amoxicillin-Clavulinic acid combination. Intermediate sensitive to Cloxacillin, Ceftriaxone & Azithromycin. Staphylococcus aureus exhibit resistant to Cephradine & Cefixime [Table V]. Antimicrobial susceptibility of Pseudomonas showed a very good sensitivity to Amikacin, Imipenem, Tazobactam & Gentamycin, intermediate resistant to Colistin & Ciprofloxacin but resistant to Ceftazidime and Co-trimoxazol [Table VI]. Antimicrobial susceptibility pattern of Klebsiella was evaluated. Klebsiella exhibit sensitivity to Amikacin, Colistin, Tazobactam & Amoxiclav, intermediate resistant to Ciprofloxacin but

resistant to Cefixime, Cephradine, Ceftazidime & Cefuroxime [Table-VII]. Antimicrobial susceptibility of E.coli showed a very good sensitivity to, Imipenem, Amikacin, Amoxiclav, Ceftriaxone & Ciprofloxacin but resistant to Cefixime, Cephradine, Ceftazidime & Cefuroxime [Table-VIII].

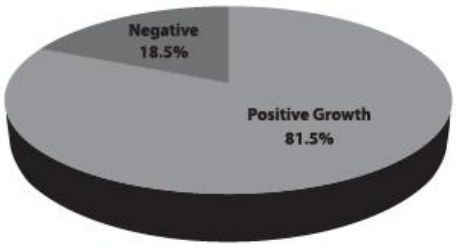

Fig-I: Percentage of Positive culture out of 200 samples.

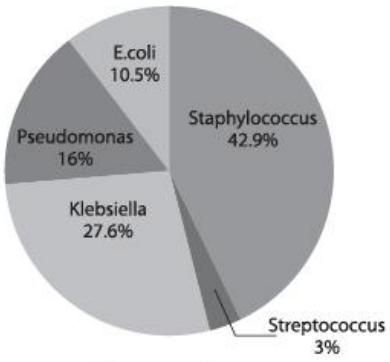

Fig-II: Types of bacteria of 163 Positive growth.

Table-I: Percentage of Positive and Negative culture out of 200 samples.

Description	Number	Percentage (%)
Total suspected samples	200	100
Positive culture	163	81.5
Negative culture	37	18.5

Table-II: Percentage of gram Positive and gram Negative bacteria out of 163 positive culture samples.

Description	Number of isolates	Percentage (%)
Total growth	163	100
Gram positive bacteria	75	46.01
Gram negative bacteria	88	53.99

Table-III: Percentage of different Gram-positive isolates out of 75 positive culture growth

Name of bacteria	Number of isolates	Percentage (%)
Total Gram-positive growth	75	100
Staphylococcus aureus	70	93.33
Streptococcus pyogenes	5	6.67

Table-IV: Percentage of different Gram-negative isolates out of 88 negative culture growth

Name of bacteria	Number of isolates	Percentage (%)
Total Gram-negative growth	88	100
Klebsiella	45	51.14
Pseudomonas	26	29.54
E. coli	17	19.32

Table-V: Antimicrobial susceptibility pattern of Staphylococcus aureus

Antimicrobial	Sensitive	Intermediate	Resistant
Vancomycin	97-99%		
Linezolid	90-92%		
Imipenem	87-90%		
Amikacin	88-90%		
Fusidic acid	84-88%		
Amoxiclav	80-81%		
Cloxacillin		50-60%	
Ceftriaxone		53-57%	
Azithromycin		40-45%	
Cephradine			83-88%
Cefixime			85-90%

Table-VI: Antimicrobial susceptibility pattern of Pseudomonas spp isolated from pus samples

Antimicrobial	Sensitive	Intermediate	Resistant
Amikacin	94-95%		
Imipenem	88-90%		
Tazobactam	78-80%		
Gentamycin	75-77%		
Colistin		68-72%	
Ciprofloxacin		63-70%	
Ceftazidime			91-93%
Cotrimoxazol			84-85%

Table-VII: Antimicrobial susceptibility pattern of Klebsiella spp

Antimicrobial	Sensitive	Intermediate	Resistant
Amikacin	93-95%		
Colistin	85-90%		
Tazobactam	86-88%		
Amoxiclav	78-81%		
Ciprofloxacin		52-55%	
Cefixime			76-82%
Cephradine			85-90%
Ceftazidime			88-93%
Cefuroxime			91-93%

Table-VIII: Antimicrobial susceptibility pattern of E. coli

Antimicrobial	Sensitive	Intermediate	Resistant
Imipenem	88-90%		
Amikacin	80-81%		
Amoxiclav	82-85%		
Ceftriaxone	77-82%		
Ciprofloxacin	73-76%		
Cefixime			76-82%
Cephradine			61-68%
Ceftazidime			66-70%
Cefuroxime			68-74%

Discussion

Pyogenic infections are still frequently seen in the developing countries and the treatment is a considerable challenge despite advances in microbiological techniques, antibiotics and surgical treatment. To ensure an adequate and efficient therapy, it is necessary to identify and treat the focus of inflammation. Management of several pyogenic infections consists of aspiration or surgical drainage followed by appropriate antibiotics. Wound infections have been a problem in the field of surgery for a long time. Advances in control of infection have not completely eradicated this problem because of the development of drug resistance. It is important to have clear conception about susceptibility and resistant pattern of organisms isolated from pus to choose the correct treatment regimen. In our study, susceptibility & resistant pattern of bacteria isolated from pus of various clinical specimens were evaluated.

A cross sectional study was carried out at the Department of Microbiology, Dhaka National Medical College & Hospital from January 2021 to July 2021. A total 200 samples were taken from various clinical specimen containing pus.

In present study, Staphylococcus aureus is the predominant organism among Gram positive organisms which is comparable with many studies.[9,10] Among gram negative bacteria, Klebsiella pneumonia is predominant organism isolated in our study with similar findings shown in studies by Sharma V et al10 and Panta K et al.[11] But in the study conducted by Kumar AR et al9 and Verma P et al,[12] it was second most common isolate.

Antimicrobial susceptibility pattern of Staphylococcus aureus was evaluated in this study. Staphylococcus aureus exhibit sensitivity to Vancomycin, Linezolid, Imipenem, Amikacin, Fusidic acid & Amoxicillin -Clavulinic acid combination. Intermediate sensitive to Cloxacillin, Ceftriaxone & Azithromycin. Highest sensitivity to Vancomycin was observed in this study & this result was consistent with study conducted by Taiwo et al.[13] Staphylococcus aureus exhibit maximum resistant to Cefixime followed by Cephradine. Resistance for third generation cephalosporin like Ceftriaxone was found in study conducted by Duggal S et al.[14] A study in Iran by sarraafzadeh F et al[15] reported 9.2% resistance for Vancomycin and some study showed 100% sensitive.[16]

Antimicrobial susceptibility of Pseudomonas showed a very good sensitivity to Amikacin, Imipenem, Tazobactam & Gentamycin but resistant to Ceftazidime and Cotrim. Pseudomonas was highly sensitive to Amikacin & highly resistant to Ceftazidime. Antimicrobial susceptibility of E.coli showed a very good sensitivity to Imipenem, Amikacin, Amoxiclav, Ceftriaxone & Ciprofloxacin but resistant to Cefixime, Cephradine, Ceftazidime & Cefuroxime . E.coli exhibit maximum sensitivity to Imipenem followed by Amikacin & showed maximum resistant to Cefixime followed by Cephradine. A study by Chaudary R et al[17] reported that Amikacin (93%) to be the drug of choice for gram negative bacterial isolates which was comparable with our study results. Similarly, Timilsina et al[18] showed the sensitivity of Amikacin to be 93.62% followed by Gentamycin 89% for gram negative

isolates. For E. coli, Timilsina et al[18] found out that the most effective antibiotic was Amikacin (100%). Shrestha et al[19] also showed Amikacin (94.38%) to be the most sensitive antibiotic for E. coli. Abdullah et al[20] showed low sensitivity of Doxycycline (11.5%) and high sensitivity to Amikacin (89.4%) in Klebsiella isolates. Antibiotic sensitivity of these microorganism showed that all of them are commonly sensitive to Amikacin.

Conclusion

In our study, the most common pyogenic organisms isolated from various clinical specimen are Staphylococcus aureus, Klebsiella spp, Pseudomonas spp, E. coli & Streptococcus pyogenes. Staphylococcus aureus is the predominant organism among Gram positive bacteria & Klebsiella spp is the predominant organism among Gram negative bacteria. This study showed that antibiotic sensitivity & resistant pattern varies from bacteria to bacteria. Amikacin is the drug of choice of both gram positive & gram negative bacteria. Based on this study, it may be concluded that testing of sensitivity & resistant pattern before starting antibiotic treatment is very important to prevent emergence of drug resistance bacterial strain. Though antimicrobial susceptibility of microorganisms varies from time to time and from place to place, so regular monitoring of bacterial susceptibility to antibiotics is essential. Antibiograms should be prepared regularly and made readily available to the clinicians to guide them in therapy. This study might be useful to revise current empirical therapy policies for treatment of bacterial infection & antibiotic therapy should be reconsidered after testing their anti-microbial susceptibility pattern.

Limitation of study: History taking of antibiotics were not possible in some case.

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