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TO IDENTIFY BACTERIAL ISOLATES IN PUS SAMPLE & STUDY THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN AT TERTIARY CARE HOSPITAL IN SOUTHERN RAJASTHAN --- AN OBSERVATIONAL STUDY

Microbiology

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ABSTRACT

OBJECTIVE: The aim of the study was to identify bacterial isolates &it's susceptibility pattern in southern Rajasthan. Archived pus culture data obtained from MBGH, RNT Medical College Rajasthan Sept.2017 to Sept.2018.

MATERIAL & METHOD: A total of 211 pus samples were included in the study, from patient attended MBGH at R.N.T. Medical College, Udaipur. Bacterial isolates obtained were identified using various phenotypic methods (Biochemical reactions& Antibiotic susceptibility test).Antibiotic susceptibility test was performed by Kirby–Bauer disc diffusion method on Mueller-Hinton Agar.

RESULT: Out of total of 211 sample; Staphylococcus aureus was most commonly isolated (24.6%) followed by Escherichia coli (17%), Pseudomonas sp. (10.8%), Methicillin sensitive staphylococcus aureus (9%), Methicillin resistant staphylococcus aureus (6%), Klebsiella (6%) among others. Among Gram positive cocci Amikacin was found to be highly sensitive and for Gram negative bacilli Meropenem was found to be highly sensitive.

CONCLUSION: There was a high resistant rate toward different antibiotic & their study give a good guide to the clinician to propose an empirical treatment for the patients. Meropenem should be kept in reserve for resistant cases.

KEYWORDS

Kirby–Bauer disc; Antibiotic susceptibility test; Meropenem

INTRODUCTION:

Pus-a whitish yellow liquid- is an amassing for body's immune system produced throughout inflammation due to bacterial or fungal infection, and consisting from protein rich liquid and dead leukocytes. Many studies, done in different areas all over the world, showed the bacterial profile and the antibiogram in their respective areas and so made an important observation for clinicians who want to prescribe empirical treatment to their patients while laboratory culture reports are awaited.[1]

Penicillin- the first antibiotic to be used on a large scale was first implicated during the World War II. In recent years, antimicrobial resistance to many humanpathogenic bacteria is being commonly reported from allover the world.

In spite of large number of newly discovered antibiotics in the recent three decades, the situation is alarming especially in the developing countries tries mainly due to of their un-systemic use. The common bacterial pathogens isolated from pus are *Staphylococcus aureus*, *Pseudomonas aeruginosa* and Escherichia coli and bacteria belonging to family Enterobacteriaceae[2].

Commonest Gram positive pathogen found in pus was Staphylococcus aureus followed by other Gram negative bacilli[3], But in India GNB was predominantly isolated compared to Gram positive pathogen[4,5].Due to increasing antibiotic resistance[4], it's difficult to treat these infections.

The indiscriminate use of antibiotics has also lead to the increase in multi-drug resistant organisms (MDRO)[3]. In the present era infections have become the leading cause of morbidity in patients of surgery, trauma etc.[4]. It is important to know the pathogens causing the infections and its antibiotic susceptibility for proper management of the patients[5].

This study was designed to evaluate theprofile of bacteria isolated from pus specimens by the using different biochemical methodsalong with their resistances pattern to different antimicrobial agents.[1]

METHOD:

Data collection

An observationalanalysis of 211 culture positive pus and tissue samples received in the department of Microbiology from various departments (orthopaedics, nephrology, plastic surgery, vascular surgery, surgical gastroenterology,cardiothoracic surgery) in the hospital between January 2017 to September 2018 was performed. Demographic data regarding the pathogen isolated and its antimicrobial susceptibility were collected and analyzed.

Sample collection, processing& culture methods:

Specimen was collected on moistened cotton swab and the swab was immersed in a container of Brain Heart Infusion(BHI) transport medium[6],the collected swab samples were inoculated on to MacConkey agar media (TM Media) at 37degreefor 18-24 hrs.andBlood agar media (Hi Media, INDIA) and chocolate agar media (Hi Media, INDIA)were incubated in a humid, 5% CO_2 atmosphere for 18–22 hours at 35 C–37 C. All the plates were incubated aerobically and initially examined for growth after 24 hrs, and the ones without growth were further incubated for up to 48 hrs.

Isolation, Identification, and Drug Susceptibility Testing of Bacteria

After obtaining pure colonies, further identificationswere done by using the standard microbiologicaltechnique, which includes Gram stain, colony morphology,and biochemical tests (MERCK and HiMedia,INDIA).

Species identification of the isolates was performed from pure colonies using classical biochemical tests according to the standard guidelines[7].

Antimicrobial susceptibility testing was carried out on each identified organism by disc diffusion method onMullerHinton agar (MHA)(Hi, Media)and blood agar (BAP) as recommended by the Clinical and Laboratory Standards Institute (CLSI)[8]. The isolates were tested against vancomycin (30 g), oxacillin (5 g), gentamicin (10 g), erythromycin (15 g), ciprofloxacin (5 g), ceftriaxone (30 g), trimethoprim-sulfamethoxazole (25 g), chloramphenicol (30 g), tetracycline (30 g), amoxicillin, ampicillin (10 g), penicillin (10 IU), and cloxacillin (5 g)[8].The zones of inhibition were measured and compared with the guidelines[8].

Quality control: The reference strains used as control were *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923)[7].

RESULTS:

Magnitude the bacteriological agents isolatedfrom pus samples:

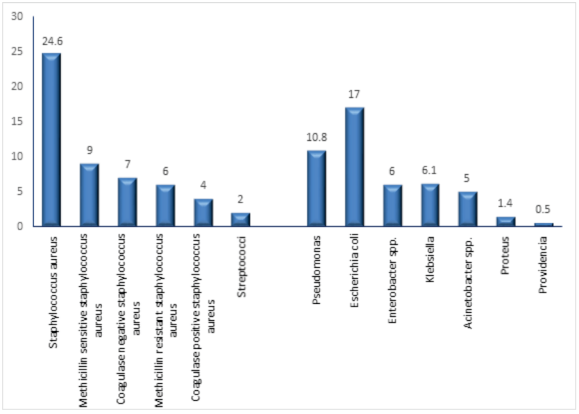
A Total of 211pus sample showing significant growthwere included in this study from Jan 2017 to Sept 2018, out of these113 (53.5%) were Gram positive bacteria and 98 (46.4%%) were Gram negative bacteria, of the isolated 98(46.4%)Gram negative bacteria,*Escherichia coli 35 (17%) was the most predominant Gram negative bacilli*followed by *pseudomonas spp. 24 (10.8%), Klebsiella spp. 13 (6%), Enterobacter spp.12(6%)Actinetobacter spp.10(5%), and* Proteus 03 (1.4%), Providencia 01 (0.5%). In another hand, of the 113isolated Gram positivebacteria,S.aureuswas the most prevalent organism 52(25%), followed by Methicillinsensitive *Staphylococcus aureus19 (9%),*

Methicillin resistant staphylococcus aureus 13 (6%), coagulase positive staphylococcus 09 (4%)and Coagulase Negative Staphylococcus 15 (7%), streptococcus 05 (2%). Our study in accordance with a similar study done by Mahat P et al (2017) in North India they observed that 71.82% were Gram-negative and 28.18% were Gram positive bacteria. In another study conducted in Nepal[10] Gram-negative bacteria were the predominant organisms isolated from wound infections. In our studythe most predominant Gram negative bacilli isolated from pus sample was Escherichia coli 17%and most predominating Gram positive bacteriaStaphylococcus aureuswas accounting.

TABLE NO. 1

ISOLATE	N	%
	Gram (+)	
Staphylococcus aureus	52	24.6
Methicillin sensitive staphylococcus aureus	19	9
Coagulase negative staphylococcus aureus	15	7
Methicillin resistant staphylococcus aureus	13	6
Coagulase positive staphylococcus aureus	09	4
Streptococci	05	2
	Gram(--)	
Pseudomonas	24	10.8
Escherichia coli	35	17
Enterobacter spp.	12	6
Klebsiella	13	6.1
Acinetobacter spp.	10	5
Proteus	3	1.4
Providencia	01	.5

Graph no. 1



Antimicrobial susceptibility patternsof bacterialisolates:-
The antimicrobial susceptibility patternsof the Gram-positive and Gram-negative bacterial isolates were presented in Table No.02.

The predominant organismStaphylococcus aureus isolate from pus cultures, showed high resistance towards most antibiotics such as ampicillin (90%), cephalosporinsand erythromycin (62%), showed resistance below 50% towards amikacin,co-trimoxazole andtetracycline(10%, 31%, 19% respectively), the second most predominant isolates*Escherichia coli, inthe study showedhighly resistance* towards to amoxicillin (83%) and amoxyclav (83%) and low level of resistance seen amikacin, gentamicin and meropenam (17.2%, 37.2%, 10% respectively) Escherichia coli and other Enterobacteriaceae are usually susceptible to meropenam, in this study Escherichia coli shown 90% susceptibility to meropenam.

In our study Methicillin sensitive staphylococcus aureusshowed high level of resistance to most of antibiotics except amikacin(0%), cefoxitin(0%) and vancomycin (5%), higher level of resistance seen for ampicillin(100%), ciprofloxacin (80%) whileasMethicillin resistant staphylococcus aureus showed relatively low resistance towards,amikacin(8%), vancomycin(15%) but highly resistance to ceftazadmine(100%), ceftriaxone(100%),amoxicillin(100%).

Table No.2

Antibiotic	S.Aureus	E.coli	Pseudomonas spp.	Enterobacter	Klebsiella	Acinetobacter
Amikacin	90%	82.8	70.8	75	23.07	70
Gentamycin	85%	62.8	66.67	41.6	30.77	60
Ceftazidime	--	5.7	37.5	16.67	15.38	100
Ceftriaxone	37%	8.6	25	33.37	15.38	70
Erythromycin	38%	--	--	--	--	--
Ampicillin	5.8%	--	--	58.33	--	10
Amoxyclav	27%	2.9	--	33.33	30.77	--
Ciprofloxacin	55.7%	11.4	54.16	66.67	23.07	70
Cotrimoxazole	69%	3	33.33	33.33	30.77	90
Piperacillintazobactum		43	75	--	30.77	80
Meropenam		90	66.67	--	23.07	--
Vancomycin	96%	--	--	67	--	--
Cefoxitin	78.8%	--	13	--	8	10
Tetracycline	80%	34.3	17	67	69.23	80

This difference in sensitivity of different microorganism to different antibiotics in the present study could be rationalized by the effect of difference in date and place on resistance. It is obvious that the antibiotic pattern and the bacterial profile of the wound infections may change from time to time and place to place. The presence of many antibiotics and their misuse, multidrug- resistant bacteria are emerging, and in our area, there are high resistance rates towards to different antibiotics.

This study recommendation to activate the role of antibiotic committee in hospital, and give good empirical guidance and antibiotic stewardship considerations.

There was a high resistant rate toward different antibiotic & their study give a good guide to the clinician to propose an empirical treatment for the patients. Meropenem should be kept in reserve for resistant cases.

DISCUSSION:-
Pus is a resultant production of many pyogenic infection, they may be endogenous as well exogenous also[10]Loss of skin integrity by various factors would provide an environment for the colonization and growth of microorganisms In a study in North India[09], they observed that 71.82% were Gram-negative and 28.18% were Gram-positive bacteria from wound infections and 63.2% of them were MDR[09]. In another study conducted in Nepal[11] Gram-negative bacteria were the predominant organisms isolated from wound infections.

In our study Gram positive cocci was predominant accounting for 53.5%recovered from most of the pus samples. Escherichia coli wasthe predominant organism isolated from pus samples as seen in several studies[4]. It is the most common Gram-negative bacilli isolated in post-operative infections[12]. In our study, there was a decrease in the no. of sensitive isolates & an increase in antibiotic resistance, among the isolates the antibiotic resistance is an increasing concern as, it's transmitted among patient which leads to difficulty in treatment. Escherichia coli being predominant in 17% of our cases. Klebsiellapneumoniae was isolated in 6% of cases, with most of them were multidrug resistant. Pseudomonas was isolated from 10.8% of our cases. The organism is mostly found in chronic infections[13].

A cine to bacter sp., the organism is present in the environment and associated with hospital acquired infections. It's a colonizer in the skin & may later leads to infection[14], by appropriate infection control measure, avoid the overuse of antibiotics are mandatory.

In our study there was an increase in the no. of Gram positive cocci throughout the study period which was mostly due to increase in the infections due to S.aureus. In a study from Bangladesh S.aureus is the commonestorganism isolated in 40.5% of the cases of wound infections[03].It's the commonest organism in most of the studies[15-16]. Staphylococcus aureusis the most common cause of abscess in25-30% of the cases, a study done by Bowler et al mostly associatedwith acutesoft tissue infections. It'sone of the cause of delayed wound infections[17].In our study Staphylococcus aureuswas found in 24.6% (52) of Gram positive bacteria, MSSA was found in 9% (19) & MRSA was found in 6%(13) of the Gram positive bacteria. All our MRSAisolates were sensitive to vancomycin. Streptococcus pyogens were isolated from 5 in our study. It's one of the frequent cause of delayedwound healing[17]. All our isolates were sensitive to penicillin, cephalosporin .In this study CONS accounted for 7% of the isolates, this finding is similar to a report in Nigeria, where Staphylococcus epidermidis accounted for 11.4%[19].CONS are ubiquitous colonizers, they are frequently found as contaminants in clinical specimens. Emergence of this organism as nosocomial pathogens, complicates laboratory interpretation of their clinical significance[19].

In our study COPS accounted for 4%of the isolates,these are the normal flora of a variety of animal species and associated with skin infections, invasive infections in immunocompromised individuals[19].

There was as light increase in the number of resistant organism through the year in our study, which may be due to the spread of the resistant genes among the organism. As our institute is a tertiary care center majority of the patients get admitted after being treated from outside hospitals where most of the patients had severe infections & they were treated with higher class of antibioticsin other hospitals which may lead to the growth of multi drug resistant pathogens.

A proper control measure, limit the over use of antibiotics & infection control procedures starting from primary health care center to tertiary level would help in the control of infection with resistant pathogen. Our study help in formulation of antibiotic policy with a reduction in rate of infections.

CONCLUSION:

- Our study gave a good overview to the clinicians about our local pus isolates bacterial resistance pattern.
- Vancomycin was found to be very effective in eradication of Gram positive bacteria (Staphylococcus aureus) isolates.
- It becomes essential to know the prevalent profile and sensitivity pattern to guide the clinicians to start the empirical treatment. Also, it is very important to establish a recommendation or good guidelines to help clinicians suggest empirical treatment.
- It has been noticed that the last resort medications of antibiotics have been implicated in empirical treatment of patients and this example why the resistance exceed the normal range towards the first line medications, but vancomycin is stile the last resort for Gram positive bacteria, and is very optimistic although increased usage.

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