International Journal of Medical and Health Research
ISSN: 2454-9142
Received: 27-05-2019; Accepted: 30-06-2019
www.medicalsciencejournal.com
Volume 5; Issue 7; July 2019; Page No. 208-212

Clinical assessment of bacteriological profile and antibiotic sensitivity patterns of aerobic pus Isolates from ANMMC Gaya

Dr. Prem Prakash[1], Dr. Deepti Kiran[2*], Dr. SP Mahto[3]
[1, 2] Tutor, Department of Microbiology, Anugrah Narayan Magadh Medical College, Gaya, Bihar, India
[3] HOD, Department of Microbiology, ANMMC, Gaya, Bihar, India
[*] Corresponding Author: Dr. Deepti Kiran

Abstract
Colonization and proliferation of bacteria in wound may lead to wound infection. Most of the latter are hospital acquired and usually following an invasive procedure or a surgical intervention. Hospital acquired infections are a world-wide in problem being an important cause of morbidity and mortality among hospitalized patients. A WHO sponsored survey showed that the prevalence of nosocomial infections was 3-21% with wound infections accounting for 5-34%. Therefore the knowledge of infectious agents causing wound infection is necessary for selection of appropriate antimicrobial therapy. Hence from the above findings the present study was planned to evaluate the Bacteriological profile and antibiotic sensitivity patterns of aerobic pus isolates in patients admitted to Anugrah Narayan Magadh Medical College, Gaya, Bihar.
The present study was planned in Department of Microbiology, Anugrah Narayan Magadh Medical College (ANMMC), Gaya, Bihar. In the present study 150 pus/wound swab samples (n=2516) received for aerobic culture & sensitivity testing from various department in ANMMC were evaluated and discussed. The study was conducted from June 2018 to April 2019.
The data generated from the present study concludes that common organisms isolated from wound infections and it helps in empirical treatment of patients based on antibiotic susceptibility patterns. So the proper care, management and implementation of infection control measures by following strict hand hygiene practices, education about the spread of bacteria through contaminated hands and environment would lead to a decrease in infections with resistant organisms which would be a burden to both the hospital and the patient.

Keywords: pus isolates, bacteriological profile, antibiotic sensitivity patterns, etc

Introduction
Antimicrobial susceptibility tests are used to determine which specific antibiotics a particular bacteria or fungus is sensitive to. Most often, this testing complements a Gram stain and culture, the results of which are obtained much sooner. Antimicrobial susceptibility tests can guide the physician in drug choice and dosage for difficult-to-treat infections [1]. Results are commonly reported as the minimal inhibitory concentration (MIC), which is the lowest concentration of drug that inhibits the growth of the organism. Reports typically contain a quantitative result in µg/mL and a qualitative interpretation. The interpretation usually categorizes each result as susceptible (S), intermediate (I), resistant (R), sensitive-dose dependent (SD), or no interpretation (NI).
The results of these tests have no true normal value. The presumed baseline would be "susceptible." However, in the era of antibiotic resistance, this is not always true. A high value means that more drug is needed to affect the organism's function or replication. A low value means that less drug is needed to affect the organism's function or replication.
The MIC is the lowest concentration of drug that inhibits the growth of the organism. Laboratories investigate this by inoculating the organism isolated from the patient into a series of tubes or cups that contain twofold dilutions of the drug. After a standardized incubation, the lowest concentration of drug that prevents visible growth of the organism is the MIC.
Susceptibility is individual to the organism tested. By itself, it does not describe presence, type, or location of infection. Culture and susceptibility should be taken under conditions that minimize contaminants. This may be under sterile conditions in an operating room or just clean conditions in a treatment room, office, or emergency department. Note that sterile conditions do not technically exist in the face of active infection. However, the goal is to collect only the organism that is causing infection. Only one sample is usually needed just before treatment is initiated. Not all organisms are easy to grow in culture, and laboratories typically take about two days to return results.
Specimens can be sent as a swab or in a sterile collection cup. If the specimen is a liquid, then it is best sent in a sterile collection cup, as the laboratory can perform more tests more readily. This needs no preservative or additional preparation for the laboratory to use it. In some cases, specimens are collected in special containers, depending on the procedure needed to collect it (eg, bronchoalveolar [BAL] traps or pleural fluid Vacutainers). These are best transferred to a standard laboratory specimen cup before being sent to the laboratory.
Blood or other bodily fluids collected with the specimen will not change or invalidate the results. Culture and susceptibility are often performed together, but not in all cases. They are also not usually included with a Gram stain. The MIC is the lowest concentration of drug that inhibits the

growth of the organism. It is garnered by inoculating the organism isolated from the patient into a series of tubes or cups that two fold dilutions of the drug. After a standardized incubation, the lowest concentration of drug that prevents visible growth of the organism is the MIC.

The disk diffusion method is a second method of determining antibiotic sensitivity. With this technique, disks impregnated with various antibiotics are placed on the surface of an agar plate that has been inoculated with the organism isolated from the patient. The antibiotic diffuses outward from the disk over a standard incubation time, and the diameter of the zone of inhibition is measured. The size of this zone is compared with standards to determine the sensitivity of the organism to the drug. A novel version of this test involves a quantitative diffusion gradient, or Epsilometer (E-test), and uses an absorbent strip with a known gradient of antibiotic concentrations along its length. When the strip is placed on the surface of an agar plate seeded with a microbe to be tested, antibiotic diffuses into the medium. The lowest concentration that inhibits growth is the MIC

For certain infections, it may be important to know the concentration of drug that actually kills the organism rather than just inhibiting its growth. This concentration, called the minimal bactericidal concentration (MBC), is determined by taking a small sample (0.01 or 0.1 mL) from the tubes used for the MIC assay and spreading it over the surface of a blood agar plate. Any organisms that were inhibited but not killed in the MIC test now have a chance to grow because the drug has been diluted significantly. After a standard incubation, the lowest concentration that has reduced the number of colonies by 99.9% is the MBC. Bactericidal antibiotics usually have an MBC equal or very similar to the MIC, whereas bacteriostatic antibiotics usually have an MBC significantly higher than the MIC.

For hard-to-grow organisms, such as obligate anaerobes, routine susceptibility testing is generally not performed. Fortunately, their sensitivities are often predictable. For severe infections caused by capable organisms, such as Staphylococcus aureus and Haemophilus influenzae, it is important to know early whether the organism is producing beta-lactamase. Therefore, rapid assays for the enzyme can determine activity in a few minutes versus around two days for a standard MIC test.

In the era of fungal antibiotic resistance, the need for testing of individual fungal isolates for susceptibility to specific antifungal agents has increased. These methods, which are used to determine the minimal fungicidal concentration (MFC), are similar to the tests for bacterial MIC. In most cases, testing must be specifically requested and may not be available at all laboratories.

Antimicrobial susceptibility is an appropriate test whenever a specimen is collected from a suspected infection site. In the face of active infection, this information, along with the Gram stain and culture, allow the physician to select an appropriate antimicrobial agent to treat an infection. The MIC and MBC can also aid in proper dosage.

In some cases, the laboratory cannot grow a pathogen, even if the sample was taken from a patient with an active infection. So, a negative culture result may not mean the patient is infection-free. In this case, a second sample may be sent. In addition, it is up to the judgement of the treating physician to choose antibiotics and treatment length when no culture or susceptibility is available.

In simple infections or infections with known pathogens, this test is unnecessary. Also, depending on the access to a lab, the complexity of the infection, or the duration of complete treatment, one may not want to send a sample for culture and susceptibility. For example, if the treatment for a common, uncomplicated UTI lasts 3 days, the results of a culture and susceptibility may not impact treatment as typical time for results are 2-5 days. This may change with new technology on the horizon. Lastly, tracking the antibiotic susceptibility for each organism allows a hospital or community to make antibiogram charts. These can guide clinicians to good initial treatment regimens.

Resistance arises through one of three mechanisms: natural resistance in certain types of bacteria, genetic mutation, or by one species acquiring resistance from another.[2] All classes of microbes can develop resistance. Fungi develop antifungal resistance. Viruses develop antiviral resistance. Protozoa develop antiprotozoal resistance, and bacteria develop antibiotic resistance. Resistance can appear spontaneously because of random mutations. However, extended use of antimicrobials appears to encourage selection for mutations which can render antimicrobials ineffective [3].

Preventive measures include only using antibiotics when needed, thereby stopping misuse of antibiotics or antimicrobials [4-5]. Narrow-spectrum antibiotics are preferred over broad-spectrum antibiotics when possible, as effectively and accurately targeting specific organisms is less likely to cause resistance, as well as side effects [6-7]. For people who take these medications at home, education about proper use is essential. Health care providers can minimize spread of resistant infections by use of proper sanitation and hygiene, including handwashing and disinfecting between patients, and should encourage the same of the patient, visitors, and family members [8].

Rising drug resistance is caused mainly by use of antimicrobials in humans and other animals, and spread of resistant strains between the two. Growing resistance has also been linked to dumping of inadequately treated effluents from the pharmaceutical industry, especially in countries where bulk drugs are manufactured [9]. Antibiotics increase selective pressure in bacterial populations, causing vulnerable bacteria to die; this increases the percentage of resistant bacteria which continue growing. Even at very low levels of antibiotic, resistant bacteria can have a growth advantage and grow faster than vulnerable bacteria [10]. With resistance to antibiotics becoming more common there is greater need for alternative treatments. Calls for new antibiotic therapies have been issued, but new drug development is becoming rarer [11].

Antimicrobial resistance is increasing globally because of greater access to antibiotic drugs in developing countries [12]. Estimates are that 700,000 to several million deaths result per year [13-14]. Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die as a result [15]. There are public calls for global collective action to address the threat that include proposals for international treaties on antimicrobial resistance [16]. Worldwide antibiotic resistance is not completely identified, but poorer countries with weaker healthcare systems are more affected.

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surgical intervention. Hospital acquired infections are a world-wide in problem being an important cause of morbidity and mortality among hospitalized patients. A WHO sponsored survey showed that the prevalence of nosocomial infections was 3-21% with wound infections accounting for 5-34% [17]. Therefore the knowledge of infectious agents causing wound infection is necessary for selection of appropriate antimicrobial therapy. Hence from the above findings the present study was planned to evaluate the Bacteriological profile and antibiotic sensitivity patterns of aerobic pus isolates in patients admitted to Anugrah Narayan Magadh Medical College, Gaya, Bihar.

Methodology

The present study was planned in Department of Microbiology, Anugrah Narayan Magadh Medical College (ANMMC), Gaya, Bihar. In the present study 150 pus/wound swab samples (n=2516) received for aerobic culture & sensitivity testing, from various department in ANMMC were evaluated and discussed. The study was conducted from June 2018 to April 2019.

These samples were processed on blood agar, chocolate agar, and MacConkey agar media and incubated at 37°C under aerobic conditions. The organisms were identified by biochemical reactions, Gram stain, and motility tests as applicable as per standard operative procedure. The antimicrobial susceptibility tests were done by Kirby–Bauer's disk diffusion method on Mueller–Hinton agar and interpreted as per Clinical Laboratory Standard Institution guidelines.

All the patients were informed consents. The aim and the objective of the present study were conveyed to them. Approval of the institutional ethical committee was taken prior to conduct of this study.

Following was the inclusion and exclusion criteria for the present study.

Inclusion criteria: Samples collected of pus/wound swab.

Exclusion criteria: Wounds which are closed and primarily healed are excluded.

Results & Discussion

Pyogenic infections are characterized by inflammation with pus formation. These infections may be endogenous or exogenous [18]. Loss of skin integrity by various factors would provide an environment for the colonization and growth of microorganisms. The growth of the pathogens depend on the type of wound such as in clean wounds the growth would be minimal where as in traumatic wounds there would be an increased chance of infection requiring an aggressive management [19-20].

In a study in North India [21], 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 [10]. In another study conducted in Nepal [22] Gram-negative bacteria were the predominant organisms isolated from wound infections.

β-lactam antibiotics are the major bulk of prescribed antibiotics in ICUs across the globe because of their efficacy, broad spectra and low toxicity [23]. However, irrational use of these antibiotics has resulted in the development and spread of drug resistant bacterial pathogens especially in the developing countries [24]. Of the particular concern, increased occurrence of Gram negative bacteria, including multidrug resistant nonfermenters (Acinetobacter baumannii and Pseudomonas species) and Enterobacteriaceae producing extended-spectrum beta-lactamase(ESBL) and carbapenemases in severe healthcare-associated infections has evolved as a significant clinical threat for medical fraternity in the recent decades [25-26]. The broad substrate profile of these enzymes may affect entire beta lactam agents, and also the organisms with these enzymes are additionally found resistant to aminoglycosides and fluoroquinolones, further compromising the therapeutic choices for severe infections in ICU [27-28].

Table 1: Age and Sex

Parameters	No. of Cases
Total	**150**
Sex:	
Males	89
Females	61
Age:	
20 – 30 years	21
31 – 40 years	48
41 – 50 years	32
51 – 60 years	24
Above 60 years	25

Table 2: Prevalence of aerobic bacterial isolates obtained from positive pus cultures

Gram positive cocci (GPC) Isolate	Number of Cases
Staphylococci aureus	31
CONS	10
Enterococcus spp.	3
Streptococcus spp.	1
Total	45
Gram negative bacilli Isolate	
Escherichia coli	45
Klebsiella pneumoniae	21
Enterobacter spp.	7
Citrobacter spp.	3
Proteus spp.	4
Pseudomonas aeruginosa	14
Acinetobacter spp.	11
Total	105

Table 3: Antibiogram enterobacteriaeace

GNB Isolated	E. coli	Klebsiella spp	Enterobacter spp	Proteus spp.
No. of Isolates	**45**	**21**	**7**	**4**
Antibiotic	**Sensitive org.**	**Sensitive org.**	**Sensitive org.**	**Sensitive org.**
Ampicillin	3	1	0	0
Gentamicin	24	4	2	3
Amikacin	32	4	3	2
Amoxy-clavulate	5	2	0	0
Cefoperazone-Sulbactam	21	2	2	2
Piperacillin-Tazobactam	16	3	2	2
Cefepime	6	3	1	1

Ceftriaxone	4	1	1	0
Ertapenem	23	4	1	0
Imipenem	30	4	2	1
Meropenem	14	5	3	2
Ciprofloxacin	4	2	1	1
Tigecycline	43	11	3	0
Trimethoprim/Sulfamethoxazole	11	1	1	1
Colistin	43	20	5	3

Table 4: Antibiogram Non fermenters

GNB Isolated	**Pseudomonas aeruginosa**	**Acinetobacter sp**
No. of isolates	14	11
Antibiotic	Sensitive org.	Sensitive org.
Ceftazidime	6	0
Gentamicin	7	1
Piperacillin-Tazobactam	5	1
Amikacin	8	1
Aztreonam	3	1
Cefoperazone-Sulbactam	7	1
Cefepime	6	0
Doripenem	7	1
Imipenem	8	1
Meropenem	7	1
Ciprofloxacin	7	1
Levofloxacin	3	2
Tigecycline	2	6
Trimethoprim/Sulfamethoxazole	12	1
Colistin	12	9

Table 5: Antibiogram Staphylococcus aureus

GPC Isolated	**Staphylococcus aureus**
No of isolates	31
Antibiotic	**Sensitive org.**
Cefoxitin	7
Erythromycin	15
Clindamycin	21
Trimethoprim/Sulphamethoxazole	9
Daptomycin	29
Linezolid	30
Teicoplanin	29
Vancomycin	30
Tetracycline	24
Tigecycline	25
Rifampicin	25
Ciprofloxacin	1
Levofloxacin	0
Gentamicin	16

Antimicrobial resistance is a recognized problem in South Asian region with high levels of resistance among Gram negative organisms reported frequently [29]. It is well known fact that resistance is due to extreme antimicrobial consumption, and overuse of antibiotics can be surmised as one of the factors contributing to the high rates of antimicrobial resistance in Nepal. In this study, many microorganisms found as resistant to different antimicrobial agents and in some cases to nearly all agents representing an alarming scenario in our intensive care setting.

The risk factors associated with infection by MDRO were commonly age, sex, previous antibiotic therapy, previous hospitalization, increased length of stay in the hospital, patient comorbidities like immunosuppression, chronic liver disease, heart disease etc. and general medical condition [30]. There was a slight increase in the number of resistant organisms through the years in our study which may be due to the spread of the resistant genes among the organisms. As our Institute is a tertiary care centre majority of the patients get admitted after being treated from outside hospitals where most of the patients had severe infections and they were treated with higher class of antibiotics in other hospitals which may lead to the growth of multidrug resistant pathogens. Proper control over usage of antibiotics and infection control measures starting from primary health care centers to tertiary levels would help in the control of infection with resistant pathogens.

Conclusion

The data generated from the present study concludes that common organisms isolated from wound infections and it helps in empirical treatment of patients based on antibiotic susceptibility patterns. So the proper care, management and implementation of infection control measures by following strict hand hygiene practices, education about the spread of bacteria through contaminated hands and environment would lead to a decrease in infections with resistant organisms which would be a burden to both the hospital and the patient.

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