STUDY ON BACTERIOLOGICAL PROFILE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS

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Abstract

The emerging trends of antibiotic resistance in widely implicated pathogens exacerbate the already high risk of bacterial infections in cancer patients. Specifically, gram-negative bacilli including E. coli, Klebsiella pneumoniae, and Acinetobacter spp. provide a greater challenge in the Indian context. It is especially worrisome because resistance is on the rise among gram-positive bacteria. The goal of this study was to keep track of the most common bacteria that cause infections in cancer patients and to describe how well antibiotics work against them.

Keywords: Bacteriological, Antibiotic, gram-positive, E. coli, Klebsiella pneumoniae, and Other Gram-Positive Bacteria

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1. Introduction

When germs are able to enter the bloodstream, a condition known as bacteremia develops. It's true that blood is naturally sterile. Therefore, any bacteria in the blood poses a risk to all of the body's organs. [1] Shockingly, most cases of occult bacteremia clear up on their own, and long-term complications are becoming increasingly rare. Multiple organ failure, septic shock, disseminated intravascular coagulation, and death are all possible outcomes of bacteremia. Invasive surgeries, prolonged survival of immunocompromised and critically ill patients, and the administration of broad-spectrum antibiotics that suppress the normal flora and allow the formation of resistant strains are all common causes of bloodstream infections. [2]

As a result of increasing improper use of antibiotics and rising resistance levels, antimicrobial resistance has emerged as one of the most pressing public health issues, especially in developing nations.[3] India has one of the world's highest rates of infectious disease burden, and a recent analysis shows that the country's healthcare system is making excessive and unjustified use of antimicrobial medicines to treat these infections. Further, it has been demonstrated that India's health sector is severely underfunded, creating an environment conducive to the emergence of drug resistance. [4] The need for more responsible antibiotic use was recently emphasised by a study that looked at India. If antibiotics no longer work, diseases in the community will be harder to manage and medical care will be less effective.
The epidemiological features of antibiotic resistance are poorly understood in the majority of South East Asian countries. Many international organisations, including the World Health Organization, the European Center for Disease Control, and resolutions from the World Health Assembly, have identified antimicrobial resistance as a serious public health concern. However, it will be difficult for policymakers and healthcare providers to address this issue. In an effort to maintain the efficacy of antimicrobial drugs in the treatment and prevention of microbial illnesses, the World Health Organization has developed a regional strategy on antimicrobial resistance. [5]From a public health perspective, it is essential to seek out the existing situational analysis in the Indian setting so that suitable interventions can be implemented at the community level to confront the problem. In light of this, the study looked at the problem load and many different parts, as well as recent progress, problems, and possible solutions to the problem of antimicrobial resistance.

The types of bacteria present and how they react to antibiotics
The skin is the body's largest organ and serves a vital function in keeping us alive by, among other things, keeping us at a comfortable internal temperature and protecting us from bacteria and other pathogens. When the epidermis is broken down, the subcutaneous tissue becomes visible. This makes for a wet, warm, and nutrient-rich environment ideal for bacterial colonisation and growth. [6]

There is a serious threat to public health from wound injuries, which make up the vast majority of trauma cases and rank among the worst types of trauma injuries. Age, gender, diabetes, stress, food, and oxygenation are just a few of the many factors that might impede the delicate process of wound healing. After skin has been punctured or otherwise compromised, most wound infections are caused by bacteria that have made their way into the affected area. [7] Pus is a localised inflammatory response composed of white blood cells, damaged cells, and dead tissue.This leads to the development of a pus pocket. Issues with the host and the microbial burden, as well as age, poor diet, obesity, metabolic or endocrine disorders,

and so on, are all potential factors. The way a wound infection spreads from there depends on how the body's immune system reacts to it. In 2015, wound sepsis affected anywhere from 10% to 33% of patients in India. Surgical site infections (SSI), sometimes known as surgical wound infections, and diabetic ulcers are just a few of the many potential triggers for wound infections. The incidence of SSI is a major source of concern for hospitals due to the high rates at which it often results in severe morbidity and mortality [8]. This is because it causes patients to spend more time in the hospital, which in turn increases the overall cost of their care. Wound infections can be caused by many different types of microorganisms, including bacteria, viruses, fungus, and parasites. Microbes can be either facultative, anaerobic, or aerobic. Staphylococcus aureus causes between 20% and 40% of all illnesses, making it the most common infectious agent.It is the most common infectious agent, and it is resistant to the drug methicillin. Pseudomonas aeruginosa is the most common pathogen after Staphylococcus aureus, accounting for 5-15 percent of infections.The next most common pathogens are Escherichia coli, Enterococcus sp., Proteus sp., and Klebsiella. The pathogenic agent, the path physiology, the pharmacokinetics, and the pharmacodynamics of medicine all play a role in determining which antibiotic will be most effective in treating an infection. Due to the ever-increasing problem of antibiotic resistance, developing efficient treatment strategies for gram-negative infections has become more challenging. The United States is a prime example of a country where antibiotics are still routinely used despite their negative effects. [9] This difficulty has grown in proportion to the magnitude of the underlying problem. In light of recent concerns about the development of antibiotic resistance, it is important to stick to the method of testing microorganisms to see if they are sensitive to antibiotics and growing them from the start to provide the right care and avoid problems in the future.

2. Materials And Methods

Ethical clearance for this study was obtained from Institutional ethical committee held at Gouri devi institute medical science, Durgapur. Blood, HVS, wounds, urine, sputum, pus, nasal and ear swabs, and other clinical specimens were collected and transferred to the laboratory aseptically in accordance with the usual protocol. Sterilized Nunc vials were used to keep all specimens, and the containers were clearly labelled to make identification a snap. Bacteria were isolated from the samples by inoculating them in nutritional broth. Between 2020 and 2021, Gouri devi institute medical science, Durgapur took 250 samples from patients who were in the hospital.

Isolation, Detection, And Identification Of Mrsa

Clinical specimens were processed in the same way as in prior research. It took 24-48 hours at 37^{o}C for the clinical samples to be inoculated into nutritional broth and streaked onto Mannitol Salt Agar (MSA) plates. Agar plates containing mannitol salt agar after 24 hours of incubation at 37^{o}C yielded colonies of yellow bacteria. It was incubated at 37^{o}C for 18 to 24 hours for the isolation of the pure and screened colonies. Incubated on blood agar plates, 1-4-mm round, convex, yellow colonies encircled by distinct hemolysis zones emerged when the incubation process was completed. Gram's staining, a motility test, a catalase test, and a coagulase test were used to make sure the bacteria were the ones they thought they were.

Gram's Staining

Gram staining was performed on a glass slide that had been thoroughly cleaned and was free of grease. A thin smear of bacterial suspension was created by

placing a loopful of culture on a glass slide. Heat fixation was applied to the glass slide containing the smear after it had dried by air. The smear was stained with a few drops of crystal violet, which was viewed for one minute before the excess stain was washed off with tap water. It was drenched for one minute with Gram's iodine and then rinsed with water. Decolorize the stain with 95 percent ethanol (30–50 seconds) after dropping the reagent drop-wise until crystal violet failed to wash from the smear.To finish, the smear was decolored and then safranine was applied for 45 seconds before being cleansed with water once more. With an oil immersion microscope objective, the slide was looked at after it had been dried, so that it could be seen.

Motility Test with a Hanging Drop

As an alternative to staining, the bacteria's motility was determined using this procedure. A spirit light was used to polish and flambé the hanging-drop slide before placing it on the table with the depression facing up. Petroleum jelly was applied to the slide's cavity and to the cover slip's four corners with a match stick. The cover slip was adorned with a loop of culture. To create a seal, the depression slide was inserted into the coverslip with the cavity pointing down and gently squeezed. The movement of the bacteria on the slide was seen using an oil-immersion objective lens to validate the motility of the bacterial isolates.

The catalase test

Staphylococci and streptococci can be distinguished using the catalase test. Byproducts of oxygen metabolism such as hydrogen peroxide are neutralised by the catalase enzyme, which is produced by bacteria that use oxygen to breathe. The quick emergence of bubbles is a telltale sign of this response. It was decided to use a "control" and a "test" set of glass test tubes that had been disinfected. Each tube was given a squirt of regular saline. Each tube was infected and thoroughly mixed with a tiny amount of overnight developed culture of bacteria using a sterile inoculation loop. Positive catalase results were confirmed by adding a few drops of 3 percent hydrogen peroxide to the tube labelled "test" and observing the production of air bubbles.

CoagulaseTest

Coagulase detection is done using this approach. The glass slide was separated into two pieces and labelled "control" and "test" before being cleaned with ethanol and sterile cotton. To each section, a few drops of deionized water were added. A few drops of blood plasma were injected into the MSA plate-derived bacterial culture through the inoculation loop. The applicator stick was sanitised and used to thoroughly emulsify the sample. Staphylococcus aureus coagulase positivity was confirmed after the bacteria clumped together on the slide for about 5 to 10 seconds.

3. Results

Table 1.Quantitative distribution of samples

Sample types	**No. of samples**	Percentage
Urine	32	12.8
Pus	105	42
Blood	39	15.6
Sputum	7	3.88
Foley’s tip	24	13.33
CVL tip	16	6.4

Endotracheal secretions	15	8.33
Bronchial fluid	12	4.8

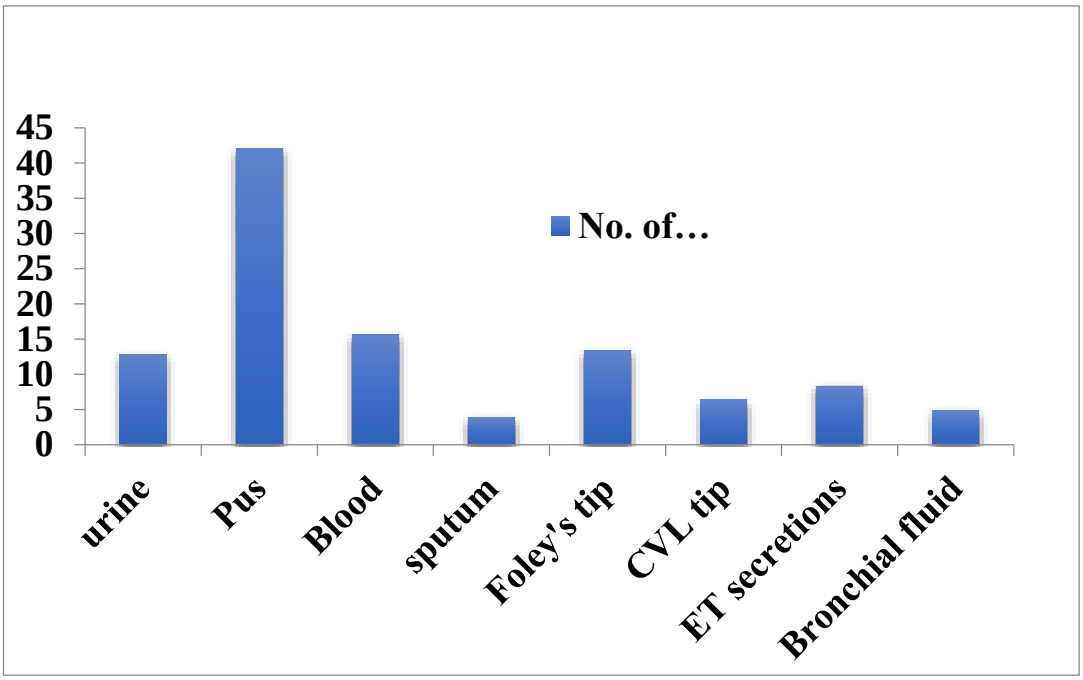

Fig 1 Quantitative distribution of samples

Table 2. Quantitative distribution of samples in different wards

Ward	ICU	Surgery	Orthopaedics	Medicine	Gynecology	Paediatrics	ENT
Samples	111	65	21	9	28	14	2

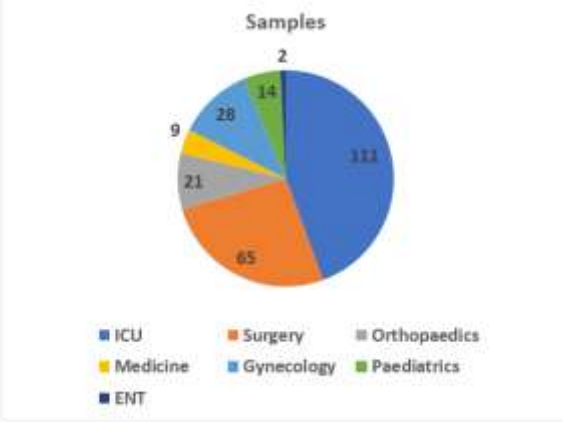

Fig. 2 Sample distribution in wards (n=250)

4. Discussions

Nosocomial infections are common, and many are caused by strains of Staphylococcus aureus that are resistant to methicillin and other medications. This gene, mecA, encodes the low-affinity penicillin-binding protein PBP 2A, which is responsible for methicillin resistance. Staphylococcal chromosomal cassette mec (SCCmec), including the mecA gene and maybe other genetic components encoding resistance to non—lactam antibiotics, can be anywhere from 21 to 60 kilobase pairs in size. Methicillin-resistant Staphylococcus aureus (MRSA) strains have been traced back to two different possible evolutionary beginnings. Based on early analyses of restriction fragment length polymorphisms obtained for MRSA isolates collected globally using probes for mecA and Tn554, the single clone hypothesis proposes that mecA entered the S. aureus population once, leading to the formation of a single MRSA clone that has since spread around the world. The second theory proposes that MRSA strains evolved multiple times through the horizontal transfer of mecA into phylogenetically distinct methicillin-susceptible S. aureus (MSSA) progenitor strains. This theory is based on the detection of mecA in diverse S. aureus multilocus enzyme electrophoresis types. DNA microarray analysis has found mecA in at least five separate lineages, suggesting that horizontal mecA transfer has been crucial to the development of MRSA. Recent in vivo evidence of mecA transfer from S. epidermidis to S. aureus suggests that mecA transfer to MSSA may be more common than previously thought.

Staphylococcus aureus can be detected in samples by looking for the presence of yellow colonies on mannitol salt agar, which are produced when the bacteria ferment the mannitol sugar. Smears of the bacterial isolates stained with Gram's stain showed colonies of gram-positive cocci. Naked oxygen is produced when a hydrogen peroxide solution is introduced into test and control tubes containing bacterial isolates. In the slide test, bacterial isolates were added to a hydrogen peroxide solution, which then created newborn oxygen. The presence of coagulase-positive S.aureus in the test sample was confirmed by seeing clumping of plasma in the test circle but not in the control circle. Coagulated plasma in the test circle and no clumping factor in the control circle indicates the presence of coagulase-positive S.aureus.

5. Conclusions

Microbial evolution and antibiotic overuse inevitably lead to the emergence of resistant microbes. The growth of MRSA in this region has been phenomenal. Methicillin-resistant Staphylococcus aureus (MRSA) has served as a prototypical multiresistant nosocomial infection for decades. It's a major cause of illness and death that affects people all around the world. In emerging countries like India, both access to healthcare and the prevalence of communitarian disease are on the rise. Variable estimates suggest MRSA prevalence in India at between 13 and 47 percent. It has a propensity to rapidly acquire resistance to cutting-edge antibiotics, both because of its increased virulence and the emergence of new antibiotic resistance genes. Despite the availability of a wide variety of medications, MRSA is the most feared multiple-antibiotic-resistant infection in ICUs. The main cause of this is the diminishing number of treatment choices for MRSA infections. Drug resistance to vancomycin and linezolid has been documented in a number of Indian cities. Research into the creation of antibiotics needs to be on par with that effort. The looming threat of drug-resistant MRSA infections necessitates the development of novel antibiotics.

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