DETECTION OF MULTIPLE DRUG RESISTANCE (MDR) BY E- TEST METHOD OF GRAM NEGATIVE BACTERIA ISOLATED FROM CLINICAL ISOLATES FROM WOUND INFECTION

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Abstract:

The purpose of this study, to evaluate the multiple drug resistance bacteria from clinical samples from wound infection E-test method. E-test used for determining the MIC value of antibiotics for the treatment of patient on what concentration of antibiotic is suitable. Infection continues to be a major complication of wounds with significant increase in costs, morbidity and potential mortality. Of these 150 samples, 133 samples showed positive results for bacteria. From 133 wound samples 149 bacteria were isolated. These bacteria were screened on different biological media for their isolation and identification. The Gram negative bacteria were isolated were P. aeruginosa (34.66%), E.coli (27.33%), P. vulgaris (16%), Klebsiella spp (13.33%), P. mirabilis (6.66%) and Serratia spp. (1.33%). These samples were tested for their antibiotic susceptibility testing and followed by MIC by using E- test. The MIC value of Piperacillin /Tazobactam was found to be 12-16mcg/ml. The MIC value of Cefriaxone and Co-trimaxazole were found to be in the range of 0.25 to 0.064mcg/ml. The most common wound infection with Cefriaxone and Co-trimaxazole were the most effective antibiotics for the treatment of resulting infections based on the culture and sensitivity results toward multidrug resistant Gram-negative.

Keywords:

E-test, Gram Negative Bacteria (GNB), Antimicrobial Resistance, Bacterial Isolates, MDR

Introduction:

Wound is a breach in the skin, and exposure of subcutaneous tissue following loss of skin integrity providing a moist, warm and nutritive environment that is conducive for colonization and proliferation of opportunistic and pathogenic microorganisms

[1]. Wound can be classified into two types, mainly open and closed wound. Open wounds include incisions, lacerations puncture wounds, gunshot

wounds and abrasions. Closed wounds include contusions more commonly known as bruises, hematomas crush injury

[2]. Most times contaminating microbes are eliminated by the host immune system and do not persist, but species that grow and divide may become established, causing wound colonization and infection. Infection in a wound delays healing and may cause wound breakdown, herniation, or complete wound dehiscence

[2]. Most post-operative wound infections are hospital acquired, and vary from one hospital to the other and are associated with complications, increased morbidity and mortality

[3,4]. The rapid and irrepressible increase in antimicrobial resistance of pathogenic bacteria is widely accepted as a major problem that has been observed over the last decade

[5]. The condition is serious in developing countries owing to irrational prescriptions of antimicrobial agents

[6]. Antimicrobial resistance can increase complications and costs associated with procedures and treatment. Antimicrobial resistance among pathogens of wound infections is on the increase.

The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to a greater incidence of infections caused by Enterobacteriaceae family. The widespread uses of antibiotics, together with the length of time over which they have been available have led to major problems of resistant organisms, contributing to morbidity and mortality. The aim of the current study is to determine the proportion of multiple drug resistance (MDR) producers among Gram Negative Bacteria isolates from clinical pathogens and their antibiotic susceptibility patterns.

Material Methods:

1. Sample Collection : During the study, a total of 150 surgical wound samples were collected from patients using sterile cotton-tipped applicators. The swabs samples were collected before wound dressing. They were inoculated

aseptically into sterile nutrient broth as transport medium and were transported to the laboratory within 48 hours for analysis. The collected samples were streaked on freshly prepared nutrient agar plates and incubated at 37℃ for 24 hours. Bacterial colonies differing in size, shape and colour were selected from the different plates and further subcultured on nutrient agar by the streak plate technique and incubated at 37℃ for 24 hours after which, were maintained on agar slants for further characterization and identification. The bacterial isolates were characterized based on colonial and cell morphology, growth on differential / selective media such as Eosin Methylene Blue, CLED, Pseudomonas isolation agar, MacConkey Agar, Phenylalanine Agar etc. Biochemical tests which include Gram's reaction, indole tests, methyl red, Voges-Proskauer, Citrate Utilization, Motility, utilization of carbohydrates such as glucose, sucrose, mannitol, lactose and fructose, oxidase, catalase, coagulase and starch hydrolysis test. The bacterial isolates were identified by comparing their characteristics with those of known taxonomy using the schemes of Cowan S. T.[7]. 2. E- test : The MIC of Imipenem (64-1mcg), Meropenem (32 - 0.002mcg), Piperacillin/Tazobactam (256 - 0.016mcg), Ampicillin (256 - 0.016mcg), Ceftazidime (32-0.002mcg), Cefriaxone (32-0.002mcg) Co-trimaxazole(32-0.002mcg) were tested by using E-test which consists of a plastic strip. Tested colonies from overnight culture were suspended with 0.85% of normal saline (NaCl) to a turbidity of 0.5 McFarland s standards. A sterile cotton swab was used to produce a uniform layer on a Mueller-Hinton agar plate and the excess moisture was allowed to be absorbed for about 15 min before the E-test MBL strip was applied. The plate was incubated for 16 to 18 h at 37° C and the MIC end points were read where the inhibition ellipses intersected the strip [8]. Table No.1 : Bacteria isolated from clinical isolates from wound infection Sr. No. Bacterial Isolates No. of Isolates Percentage 1. Psudomonas aeruginosa 52 34.66 2. E. coli 41 27.33 3. Proteus vulgaris 24 16.00 4. Klebsiella pneumoniae 20 13.33 5. Proteus mirabilis 10 6.66 6. Serratia marcescens 02 1.33 Total 149 100%

Result and Discussion:

Of these 150 samples, 133 samples showed positive results for bacteria. From 133 wound samples 149 bacteria were isolated. Some sample gave more than two bacteria from wound samples. A total of 150 wound samples were collected from different hospital of the city. They were screened on different biological media for their isolation and identification. After identification it was found that Gram negative bacteria like P. aeruginosa (34.66%), E. coli (27.33%), P. vulgaris (16%), Klebsiella spp. (13.33%), P. mirabilis (6.66%) and Serratia spp. (1.33%) were isolated from clinical samples [Table No. 1, Fig .1]. These samples were tested for their antibiotic susceptibility testing and followed by MIC by using E- test. Meropenem, Imipenem and Amplicillin showed highest resistance against all tested bacteria. Imipenem showed highest resistance against E. coli (92.68%) and P. aeruginosa (90.38%) [Table No. 2, Fig. 2]. Such high antimicrobial resistance is probably promoted due to selective pressure exerted on bacteria due to numerous reasons like non adherence to hospital antibiotic policy, and excessive and indiscriminate use of broad-spectrum antibiotics. Piperacillin/Tazobactam combination also found to be resistance against tested bacteria. The MIC value of Piperacillin/Tazobactam was found to be 12-16mcg/ml. Most of the antibiotics were resistant to tested bacteria. The MIC value of Cefriaxone and Co-trimaxazole were found to be in the range of 0.25 to 0.064mcg/ml. From our results it was found that Cefriaxone and Co-trimaxazole were the most effective antibiotics toward multidrug resistant Gram-negative. The wound is considered one of the major health problems in the world, and infection is one of the most frequent and severe complications in patients who have sustained wounds. The result of this research showed that the prevalence rate of P. aeruginosa was 34.66%, in hospitals, this bacterium is a common cause of wound infections, especially of thermal burns, this is because burns have large exposed areas of dead tissue free of any defences and, therefore, are ideal sites for infection by bacteria from the environment or normal microbiota.[9] This finding is in contrast with the work done by

Nwachukwu et al., (2009) [10] in Abia state, Nigeria who reported 32.90% prevalence rate of P. aeruginosa. In our study, 34.66% prevalence of Pseudomonas spp. was found. It is not surprising that the two microorganisms most frequently isolated from the wound samples in the retrospective study were P. aeruginosa and E. coli. The frequent occurrence of Gram negative organisms has also been reported by Sani et al., 2012 [11]. Further analysis of the retrospective studies also showed that Ps. aeruginosa, E. coli and Pr. mirabilis are associated with surgical site infections. This finding is similar to that reported by Nwachukwu et al., 2009 [12] who found that 21.3, 19 and 10.9% were E. coli, Pr. mirabilis and P. aeruginosa, respectively. The relatively high number of Enterobacteriaceae isolated in this study points to the fact that the presence of enteric organisms in the wounds at operation probably resulted to subsequent sepsis. This finding, therefore, infers that enteric organisms are important determinants of healing in surgical wounds. The incidence of the enteric bacteria also confirms the observation that most wound infections arising from abdominal procedures are presently acquired from the patient's own faecal flora (Jonathan et al., 2008) [13]. Although, several antibiotics were in use, based largely on the bacteria isolated from the wound sites; it has been suggested that treatment should be based on the patient as a whole and not the infection alone, and that management strategies must be based on data derived from a holistic assessment of the needs of the individual (Collier, 2003). Thus, routine microbiological surveillance and careful in vitro testing prior to antibiotic use and strict adherence to hospital antibiotic policy may help in the prevention and treatment of multi-drug resistant pathogens in wound infection. Klebsiella spp. Proteus spp. E.coli Pseudomonas spp.

Conclusion:

The routine microbiological surveillance and careful in vitro testing prior to antibiotic use and strict adherence to hospital antibiotic policy may help in the prevention and treatment of multi-drug resistant pathogens in wound infection.

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