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Anti-Microbial Susceptibility of Bacteria that cause wound Infection in the Surgical patents at Aguata Diocesan Hospital Umunze.

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Abstract

Prevalence of wound infection among surgical patients in Aguata Diocesan Hospital Umunze, Anambra State was investigated. Twenty (20) samples of wound were analyzed of bacteria causing wound infection. Isolation and identification of bacteria were done using spread plate method, gram stain, standard biochemical tests and susceptibility test by disc diffusion. The result showed that S.aureus, Psuedomonas aeruginosa, Klebsiella pneumonia and E.coli were isolated. The most prevalent was S.aureus followed by Psueclomonas spp, Klebsiella pneumonia and E.coli. Staphylococcus aureus was highly sensitive to centriaxone, Escherichia coli were susceptible to ciprofloxacin, Psuedomonas Pneumonia was highly susceptible to ciprofloxacin and Klebsiella Pneumonia was sensitive to all the antibiotics tested. Therefore, the study concluded that prevalence of wound infection in Aguata Diocesan Hospital, Umunze is high and can be treated with -ciprarofloxacin or by use of combined drugs which appeared to be very effective in inhibiting the causative organisms incriminated. Antibiotics sensitivity should regularly be carried out to enhance rational use of antibiotics. Treatment guidelines for use of Antibiotics should be formulated based on hospital formula and Sensitivity pattern.

Keyword: Wound, Ciprarofloxacin, Infection, Organisms, Antibiotics, Sensitivity Pattern.

Introduction

Wound infections can be prevented by restoring blood circulation as soon as possible, relieving pain, maintaining normal body temperature, avoiding tourniquets, performing surgical toilet and debridement of the wound as soon as

possible, administration of antibiotic prophylaxis for deep wound and high risk infections (WHO). High risk wounds include contaminated wounds, penetrating wounds, abdominal trauma, compound fractures, wounds with devitalized tissue; high risk anatomical sites such as hands and feet. Antibiotic prophylaxis should be started two hours before the surgical procedures. Establishment of the causative microorganism is important and treatment should be initiated based on the bacterial sensitivity patterns. Topical silver dressings have been used to treat infected wounds however; there is no evidence for their efficacy due children, this impacts negatively on the quality of life at a tender age. The antibiotic sensitivity patterns of organisms isolated from wound have not been studied fully especially in the surgical patients at Aguata Diocesan Hospital Umunze. Inappropriate antimicrobial use is associated with increased resistance Cusini et al., (2010). It was therefore important to identify the causative organisms and determine the antimicrobial sensitivity patterns to help reduce infections and ensure appropriate use of antimicrobials.

Objective of the study

Wound infection is a common problem in adults, proper management with appropriate antibiotics is therefore important to reduce morbidity that may arise. This study is aimed at determining the antimicrobial sensitivity patterns of bacteria that cause wound infections in the surgical patients at Aguata Diocesan hospital, Umunze. Therefore, the study will specifically tilt to:

- Identify the bacteria that cause wound infection.
- Determine the Prevalence of wound infection in the surgical.
- Determine the antimicrobial sensitivity patterns of the isolated bacteria.

Materials and Methods

Some materials used include: Sterile culture bottles, pipette, cotton wool, conical flask, petri dish, test tube, glass slide, bursen burner, wire loop, crystal violet, alcohol, MacConkey agar, blood agar, chocolate agar.

Specimen Collection:

Twenty (20) samples was collected from the Aguata Diocesan Hospital surgical ward, each wound specimen was collected using sterile cotton swabs which was first soaked in sterile normal saline and were used to clean the surface of the wound. The samples was collected before wound cleaning, labeled and delivered to the laboratory within one hour for

bacteriological examination. Precautions were taken to avoid cross contamination at all stages.

Sterilization of materials:

Petri dishes, media and test tubes were sterilized by autoclaving at 121°C for l5minutes at l5Psi pressure. Inoculating wire loop and pipettes were sterilized by direct flaming. Tables, incubator etc. were sterilized by swabbing with 70% of ethanol.

Media preparation

i. Preparation of MacConkey Agar:

About 4.Og of MacConkey agar (with salt) was weighed and suspended in 200m1 of distilled water in a conical flask. The suspension was heated to dissolve. The mouth of the flask was plugged with cotton wool and then wrapped with aluminum foil. It was autoclaved at 121C for l5minutes at 15 psi pressure and was allowed to cool at about 45CC before dispensing into petri dishes.

ii. Preparation of Chocolate Agar:

About S, 4g ‘:1 chocolate agar was weighed and suspended into 3ei) of distilled water in a conical flask and properly shakes dtissolve. The mouth of the flask was plugged with coi, and then wrapped with aluminum foil. It was autdiaefd at 121°C for l5minutes at 15 psi pressure and was allowed to cool at about 45°C before dispensing into plates for inoculations.

iii. Preparation of Blood Agar:

About 28g of nutrient agar powder were suspended in 1 liter of distilled water. The suspension was stirred during heating to fully dissolve all components. It was autoclaved at 12 1°C for 15 minutes and 15 and allowed to cool to about 45°C. Five percent (5%) of sterile defibrinated blood that has been warmed to room temperature were added and mixed gently. It was dispensed into sterile plates

Inoculation and incubation

At the laboratory, the culture media was prepared and poured in petri dishes up to a depth of 7mm then allowed to cool. The inoculnms ;were applied to a small area then spread using a sterile L-shaped glass rod (spread plate method). Blood agar, Chocolate blood agar, MacConkey media were used to the manufacturers’ instruction. All inocuilateci plates were labeled and incubated at 37°C for 24 ours for the organisms to grow. Kirby Bauer Disc Diffusion sensitivity test were used to determine sensitivity patterns. Appropriate sensitivity discs were placed on the inoculated plates and the drug activity was shown by zones of inhibition of growth around the discs. The diameter of the zones

were compared to a standard and categorized as resistant or sensitive (Elmer, 2005) .The drugs that were tested include: amoxicillin elavulanate, cefuroxime, ceftriaxone, imipenem, ciproflóxacin, cefoxitin, cloxacillin and ceftazidime

Sub-culture

The colonies that appeared after incubation were transferred into another fresh medium in order to obtain pure culture. It was incubated upside down to prevent contamination and condensations for another 24hours at 37 L C before a pure culture were obtained.

Isolation of Pure Culture

After the sub culturing to pure culture, biochemical test were performed. This was done by seeding the inoculums onto the agar plate and carefully streaked with wire loop and stored inside refrigerator at 5 D C. This was done to spread the inoculums in a gradient of decreasing concentration so that bacteria are ultimately deposited slightly. (Uwaezuoke, 2010).

Identification of Isolates

All the isolates were characterized by standard techniques, the identification procedures includes, conical appearance, morphology, culture on selective media and specific biochemical tests namely: gram stain, indole, catalase, coagulase, oxidase, voges-proskeur tests.

Biochemical test:

1. Gram stain

i. This was used to differentiate Gram positive (appears purple) and Gram negative (appears pink) bacteria. The following steps were followed.

ii. The dried smear was fixed by passing over a flame three times.

iii. The fixed smear was covered with crystal violet for 30-60 seconds.

iv. The stain was rapidly washed with clean water. v. The water was tipped off and the smear covered with grams iodine.

vi. The iodine was washed with clean water.

vii. The smear was decolorized rapidly (in a few seconds) with acetone alcohol, then washed with clean water.

viii. The smear was covered with neutral red stain for two minutes.

ix. The stain was washed off with clean water.

x. The back of the slide was wiped clear and placed in a draining rack for the smear to air dry.

The smear was examined microscopically first with 40x objective to check the

staining and see the distribution of materials and then in oil immersion objective to look for bacteria.

Indole test

This was used to identify Enterobacteria. Most strains of Enterobacteria break down the amino acid, tryptophan with the release of in dole.

A sterile straight wire was used, Five (5) ml of sterile tryptone water was inoculated with test organism. An indole paper strip was placed in the neck of the tube and stopper. Incubation was done at 37E1C overnight. Indole was tested by adding about O.5m1 of Kovac's reagent, the mixture was gently shaken

Red surface layer indicates positive indole test while no red surface layer indicates negative indole test.

Catalase test

This was used to differentiate the bacteria that produce the enzyme catalase such as Staphylococci from non-catalase producing bacteria such as Streptococci.

i.Two (2) ml of hydrogen peroxide solution was poured into a test tube.

ii.A wooden stick were used to remove several colonies of the tested organisms and immersed in the hydrogen peroxide solution.

iii. Active bubbling indicated a positive catalase test.

Control:

Instead of adding two drops of hydrogen peroxide, 2 drops of water was added.

Coagulase test

This test was used to identify Staphylococcus aureus which produces coagulase.

I. Tube test (detects free coagulase)
II. Plasma was diluted in the ratio of 1:10.
III. Three small test tubes was availed and labeled; test organism, positive control and negative control.
IV. 0.5m1 of the diluted plasma was pipetted into each tube.
V. Five drops (0. imi) of the test organism was added into the labeled positive and 5drops of the Staphylococcus aureus culture were added to the tube labeled positive and 5 drops of sterile broth in the tube labeled negative.
VI. The tubes were incubated at 35-37 D C after mixing gently. Clotting occurred after one hour.
VII. Clotting indicated the presence of coagulase positive organism.

Oxidase test

This test was used to identify oxidase producing organism.

1) A strip of filter paper was placed in a petri dish and soaked with 2-3 drops of freshly prepared oxidase reagents.

2) A glass rod was used to pick a colony of the test organism and smeared on the filter paper.

3) Development of blue- purple colour within a few seconds indicated positive oxidase test while no blue-purple

colour within few seconds indicates negative oxidase test.

Control:

To oxidase reagent,2 drops of water was added.

Voges- proskeur v-p) test

I. About 2m1 of sterile glucose phosphate peptone water was inoculated with the test organism and incubated at 35-37°C for 48hours.
II. A small amount of creatinine was added and mixed well.
III. About 3m1 of sodium hydroxide was added and mixed well.
IV. The bottle cap was removed and left for one hour at room temperature.
V. v. Development of pink color indicated positive test for Vogespro skeur.

Antimicrobial sensitivity testing- Disc diffusion method.

1) A sterile wire loop was used touch 3-5 well isolated colonies of similar appearance of test organism and emulsified in 3-4m1 of sterile physiological saline or nutrient broth.

2) In a good light match the turbidity of the suspension to the turbidity of the McFarland standard.

3) A sterile swab was used to inoculate a plate of Mueller Hinton agal- by spread plate method. Removed excess fluid by rotating and pressing the swab against the side of the tube above the level of the suspension. The swab was spread evenly over the surface of the medium in three directions, rotating the plate approximately 6OEC to ensure even distribution.

4) With the petri dish lid in place, the inoculums were allowed to about 3-5 minutes for the surface of the agar to dry.

5) A sterile forceps were used to place commercially prepared antibiotics disc, evenly distributed on the inoculated plate.

Each disc was lightly pressed down to ensure its contact with the agar.

6) Within 3Ominutes of applying the disc, the plate was inverted and incubated aerobically at 37E1C for 24 hours.

7) A ruler was used on the underside of the plate to measure the diameter of each zone of inhibition in mm.

Interpretation of zone sizes

Using the interpretative chart, the zones of each antimicrobial were interpreted reporting each organism as Resistant or Susceptible.

Results

Out of 20 wound samples examined, four bacteria isolates were isolated and they are Staphylococcus aureus, Pseudo monas aeruginosa, Kiebsiella spp and Escherichia coli

Antimicrobial susceptibility of isolates to antibiotic disc

Bacteria

Susceptible

1. Staphylococcuscwreus Cef, Ce, Cip,Clo ,Am, Ceft,Im , C

2. Psuedomonas aerugnosa Cip,Im,Cef,Ce

3. Kiebsiella pnuemonicz Cip,Am, Cef, Ce,Im,Ceft, C

4. Escherichia coli Cip

Keys:

Cef = Ceftriaxone

Ce Cefoxitim

Cip Ciprofloxacin

Clo = CJ.oxacillin

Am = Amoxicillin clavulanate

Ceft Ceftazidim

C Cefuroxime

Tm = Imipenem

Discussion

In this study, out of 20 wound samples collected from surgical patients in Aguata Diocesan Hospital, Umunze, the pattern of bacterial pathogens causing wound infection showed that Staphylococcus aureus was most prevalent followed by Psueclomonas aeruginosa, Kiebsiella pneumonia and Escherichia coli. The prevalence of wounds that were infected was at 82%, which is higher compared to other studies (Ahmed 2012,Cruse 1980, Crusel992 and Culver et al.,1991) but similar to others (Ameh et al.,2009). Despite use of antibiotic prophylaxis, the prevalence still remains high. Factors that could play a role in persistent wound infection are incorrect choice and dose of drugs. This finding is consistent with other studies (Giacometti et aL, 2000, Dia ci al.,

2000 and Kitara,201 1). Staphylococcus aureus was predominant and half of the isolates were MRSA which is similar to other findings (Voss 1995, Ahmed 2012,Giacometti et a.l,2000 and Cercenado 2008). Polymicrobial wound infection which has shown increasing prevalence and growing resistance to antimicrobial agents and ability to delay wound healing were seen. Wounds are always colonized by aerobic and other bacterial and fungal strains mostly belonging to the microbiota of the surrounding skin and external environment (Bowler 200land Pantosti,2007). In this study, Staphylococcus aureus showed highest sensitivity with ceftriaxone followed by cefoxitim, ciprofioxacin, cloxacillin, amoxicillin clavulanate, imipenem, cefuroxime and ceftazidime showed sensitivity below 50% which is consistent with other findings (Giacometti et al. ,2000). Escherichla coil showed highest sensitivity with ciprofloxacin, comparable to other studies (Karimi,2008 ,Kibret 2010 and Drago,2010). Absolute resistance was seen with amoxicillin clavulanate, cefuroxime, ceftriaxone, imipenem and ceftazidime, similar to other findings (Ibrahim,2012). E. coli is a facultative gram negative anacrobe commonly found in the gastrointestinal tract, due to frequent exposure to antimicrobials, resistance has emerged over time. Kiebsiella spp, showed highest sensitivity to ciprofloxacin but above average sensitivity to augmentin, cefuroxime, cefriaxone,imipenem, cefoxitin and ceftazidime which is consistent with other studies (Stock 200 1,Tansarli 2013 and http://medicine.medscape.com , 2014), in which Kiebsielict spp species were found to be sensitive to penicillin, cephalosporins, quinolones, trimethoprim and cotrimoxazole. The use of broad spectrum antibiotics has led to development of multidrug resistant strains that produce extended- spectrum beta—lactamase (John,2007).

Highest sensitivity of drug to Psuedomonas aeruginosa was seen with ciprofloxacin, followed by imipenem. Highest resistance to ceftriaxone followed by ceftazidime was seen. Studies have shown that most Pseudomonas aeruginosa isolates are sensitive to piperacillin, ceftazidime and imipenem (Giacometti et al 2000, Sevanan 2011, European journal 2013). The decreased sensitivity to these drugs is due to antibiotic overuse• and inappropriate use. Ciprofloxacin showed high. ‘in vitro’ activity.

The most effective antibiotics in this study was ciprofloxacin, this is because it showed higher zones of inhibition. Other antibiotics

like ceftriaxone and amoxicillin clavulanate were also effective on gram positive organisms, while other antibiotics used like ceftazidime, imipenem and cefoxitim are less effective of some of the antibiotics used in the study may be as a result of indiscriminate use of these antibiotics and may have been abused. This can also explain by the long term period for which those drugs have been available or in use for wound infections. These findings were in agreement with the work of (Obasekkiebor et aL,2009 and Akerele et al.,2010)

The ciprofloxacin drugs appears as promising therapeutic agents for the treatment of wound infections. Also contamination therapy of aminoglycoside with penicillin for the treatment of wound infection is promising as this may lead to increased activities of the drug. The study have showed that susceptibility testing of antibiotics is necessary to obtain sensitivity report before initiating antibiotics treatment in case of suspected wound infection. However, the decision to use a particular antibiotics depends on its toxicity, cost and mode of action.

Conclusion

Since Staphylococcus aureus, Pseudornonas aeruginosa, Escherichia coli and Kiebsiella pnuemonia were involved in wound infection, ciprofloxacin or use of combined drugs should be used for therapeutic purpose of wound infection.

Recommendation

1. Due to high resistance of the organisms to antibiotics, sensitivity tests should be regularly carried out to enhance rational use of antibiotics and antibiotic choice should be made based on the sensitivity patterns.

2. Treatment guidelines for use of antibiotics should be formulated based on the hospital formular and the sensitivity patterns. This should be reviewed occasionally to ensure rational use of antibiotics.

References

Chow, H. H., Hakim, I. A., Vining, D. R., Crowell, J. A., Tome, M. E., Ranger-Moore, J.,

Cordova, C. A., and Mikhael, D. M. (2007). Modulation of Human Glutathione S-

Transferases by Polyphenol.. *Cancer Epidemiology Biomarkers & prevention*, **16**(8):6-8.

Clementi, E., Brown,A., Guy, C., Feelisch, M., Moncada, S., Bugg, S., Oconnell, M.J,

Goldsbrough,P.B. and Cobbett, C.S. (1999). "Phytochelatin synthase genes from the

Arabidopsis and the yeast *Schizosaccharomycespombe*" *Plant cell*, **11** (6): 1153–64.

.

Enlander, C and Derek, O.(2002). "CFS Treatment using Glutathione in Immunoprop". *The CFS HandBook.* **8**(2)**:**58-62.

Fernández, A., and Scott. R. (2003). "Dehydron a structurally encoded signal for protein interation" *Biophysical Journal* ,**85** (3): 1914–28.

Grey, V., Mohammed, S. R., Smountas, A. A., Bahlool, R., and Lands, L. C. (2003). whey Improved glutathione status in young adult patients with cystic fibrosis supplemented with protein. *Journal of Cystic Fibrosis*, **2** (4): 195–8.

Gross,W Clark L., Innace,O Joy K., Hovatter,K., Renee C.,Meier,O., Henry,L., Smith,K and William, J.(1993). "Biochemical manipulation of intracellular glutathione levels influences cytotoxicity to isolated human lymphocytes by sulfur mustard". *Cell Biology and Toxicology* **9** (3): 259–67.

Herzenberg, L. A., De Rosa, S. C., Dubs, J.G., Roederer, M., Anderson, M. T., Ela, S .W. and Deresinski, S. (1997). "Glutathione deficiency associated with impaired survival in HIV disease" . *Proceedings of the National Academy of Sciences* **94** (5): 1967–72.

Meyer, K.,Andreas, J., Brach, T., Marty, L., Kreye, S., Rouhier, N., Jacquot, J., Hell, Oand RüDiger, E. (2007). "Redox-sensitive GFP in Arabidopsis thalianais a quantitative biosensor for the Redox potential of the cellular glutathione redox buffer". *The Plant Journal* ,**52** (5): 78-86.

Meyer,K., Andreas J., May, B., Mike J., Fricker,W. and Mark,H. (2001). "Quantitative in vivo measurement of glutathione in Arabidopsis cells". *The Plant Journal* **27** (1): 67–78.

Micke, P., Beeh, K. M. and Buhl, R. (2002). "Effects of long-term supplementation with whey proteins on plasma glutathione levels of HIV-infected patients". *European Journal of Nutrition***, 41** (1): 12–18

Micke, P., Beeh, K. M., Schlaak, J. F. and Buhl, R. (2001). "Oral supplementation with whey proteins increases plasma glutathione levels of HIV-infected patients". *European Journal of Clinical Investigation* **31** (2): 171–178.

Nelson, D. L., and Cox, M.M. (2005). *Lehninger's Principles of Biochemistry* (4th ed.). New York, New York: W. H. Freeman and Company.

Parisy, V., Poinssot, B., Owsianowski, L., Buchala, A., Glazebrook, J., Mauch, F. (2006). "Identification of PAD2 as a γ-glutamylcysteine synthetase highlights the importance of glutathione in disease resistance of Arabidopsis". *The Plant Journal* **49** (1): 159–172.

Rouhier, N., Lemaire, S. D. and Jacquot, J. (2008). "The Role of Glutathione in Photo Organisms, Emerging Functions for Glutaredoxins and Glutathionylation". *Annual Review Of* synthetic *Plant Biology*,**59**(8):143-152.

Albano, O. (1989). "Effects of Oral S-Adenosyl-l-Methionine on Hepatic Glutathione in Patients with Liver Disease". *Scandinavian Journal of Gastroenterology*, **24** (4): 407-412.

Walker, J. H. And Wilson, K. (2000). *Principles and Techniques of Practical Biochemistry*. Cambridge, UK: Cambridge University Press. pp. 287–289. ISBN 0-521-65873-X.

Witschi, A., Reddy, S., Stofer, B., and Lauterburg, B. H. (1992). "The systemic availability of oral glutathione", *European Journal of Clinical Pharmacology* **43** (6): 667–672.