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ISOLATION, CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY STUDIES OF *Streptococcus pneumoniae* FROM MALIGNANT ULCERS AT AHMADU BELLO UNIVERSITY, ZARIA

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ABSTRACT

The isolation, characterization and antibiotic susceptibility studies of *Streptococcus pneumoniae* strains in malignant ulcers were carried out. The total number of samples that was collected from patients from March to August 2007 amounts to One hundred and twenty (120). Eleven (11) different strains of *S. pneumoniae* were isolated, identified using conventional methods, the antibiotic susceptibility pattern of the isolates and their percentage susceptibility to the different antibiotics was also determined. The Breast and the skin has the highest number of isolates of 5 (45.5%) each. The isolates were 100% susceptible to ciprofloxacin, but were resistant to ampiclox and amoxicillin. The pattern of susceptibility to the antibiotics common to the isolates is ciprofloxacin, streptomycin, pefloxacin and gentamicin. Ciprofloxacin, gentamicin, pefloxacin and streptomycin could serve as potent antimicrobial agent against *S. pneumoniae* in the study area as revealed by this investigation.

KEY WORDS: Antibiotic, Susceptibility, *Streptococcus, Pneumoniae*, malignant, ulcers

Streptococcus pneumoniae is Gram-positive, alpha-hemolytic, bile-soluble and anaerobic member of the genus streptococcus (Ryan and Ray, 2004). It is a significant human pathogenic bacterium and is the subject of many humoral immunity studies. The name of this organism notwithstanding, it causes many types of *pneumococcal* infections apart from pneumonia, these includes acute sinusitis, otitis media, meningitis, bacteremia, sepsis, osteomylitis, septic arthritis, endocarditis, pericarditis and cellulites and have been reported to be the common cause of bacterial meningitis in adult, children and one of the top two isolates found in ear infection (Dagan, 2000).

S. pneumoniae can be differentiated from *Streptococcus viridians*, some of which are also alpha-hemolytic, using an optochin test, as *S.pneumoniae* is optochin-sensitive. *S. pneumoniae* can also be distinguished based on its sensitivity to lysis by bile. The highest rate of invasive pneumococcal disease (IPD) are seen in children under two years of age, in whom it is currently the leading cause of invasive bacterial disease in Canada and the United States (Bjornson *et al*, 2000). IPD is also a leading cause of illness and death among the elderly and persons having underlying chronic medical conditions (CDC, 1997).

A report of resistance to drugs such as erythromycin, tetracycline and chloramphenicol has been reported (Cybulaka et al., 1970). Although effective antimicrobial drugs have reduced case of fatality, *S. pneumoniae* remains a leading global cause of morbidity and mortality (Hanasman, and Bullen,1967; Mandell et al., 2000). This study was conducted to determine whether and to what degree pneumococcal isolates collected were susceptible to various antimicrobial agents used in the study.

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MATERIALS AND METHODS

Swab Sampling

Swab samples were collected from one hundred and twenty (120) patients with malignant ulcers attending Radiotherapy and Oncology, Maxillo-facial and surgical Out-patient Department Clinics at Ahmadu Bello University Teaching Hospital, Zaria, Nigeria, from March and August, 2007 for the isolation and characterization of the bacterial contents. Prior to the collection of the swabs, the wound surface and the bed were cleansed with sterile saline. The swabs were immediately placed in bijou bottles containing sterile peptone water for transfer of the swab samples to the laboratory for culturing within 24 hours of collection.

Isolation and Characterization

The bacterial isolation was done on Blood agar , Nutrient agar and Chocolate agar media. The plates were incubated at 37°C, and left for 48 hours, but were inspected daily. The identification of the isolates was done by the examination of a range of phenotypic properties, such as colony morphology and gram staining. Other Biochemical parameters used in the identification include: catalase test, Hemolysis, Bile Solubility and Optochin sensitivity test. All the tests were carried out in accordance with the standard methods (Chessbrough, 2002).

Antibiotic Susceptibility Testing

Suspension of the isolates were prepared into clean Sterilized tubes according to 0.5 MacFarlane's standard using *Staphylococcus aureus* ATCC 25923 as a positive control strain, before plating on Muller-Hinton agar plates. The following antibiotics (Abtek Biological, UK), Ciprofloxacin (10 µg), gentamicin (10 µg), cefuroxime (20 µg), amoxacillin (30 µg), Ceftrixone (25 µg), Pefloxacin (10 µg), gentamicin (10 µg), ampiclox (30 µg), co-trimoxzazole (30 µg) and Erythromycin (10 µg) were gently placed on the agar plates to ensure contact. The plates were incubated at 37°C for 18-24 hours. The zones of inhibition measured after incubation to the nearest millimeter were interpreted using the chart adopted from National Committee for Clinical Laboratory Standards (NCCLS, 2002).

RESULTS

Table1: *Streptococcus pneumoniae* isolated from different sits of infection in Ahmadu Bello University Teaching Hospital, Zaria

Site of Infection	*Streptococcus pneumoniae* (n = 11)	% rate of Isolates
Breast	5	45.5
Skin	5	45.5
Mouth	0	0.0
Nostril	0	0.0
Ear	1	9.0
Antrum	0	0.0
Tongue	0	0.0
Total number of Isolate	11	100.0

n = number of isolates of *Streptococcus pneumoniae*.

Table ,1 shows the occurrence of the *Streptococcus pneumoniae* isolates on the different sites of infection. The breast and the skin have five (5) isolates each, while the ear has only one (1) isolate. No isolate was found on the other sites of infection.

Table 2: Antibiotic Susceptibility studies of Streptococcus pneumoniae indicating percentage resistance, intermediate and susceptibility of the isolates to the antibiotic

Antibiotics	Resistance (%)	Intermediate (%)	Susceptibility (%)
Ciprofloxacin	0	0	100
Streptomycin	27	9	64
Co-timoxazole	45	19	36
Erythromycin	82	0	18
Perfloxacin	9	9	82
Gentamicin	9	9	82
Ampiclox	91	0	9
Cefuroxime	55	18	27
Amoxacillin	91	9	0
Ceftriaxone	27	28	45

Table,2 above reveals the degree of sensitivity of *Streptococcus pneumoniae* isolates to the antibiotics used. Ciprofloxacin showed 100% efficiency against the isolates, while gentamicin and pefloxacin were also highly effective against the isolates. However, the isolates strongly resisted ampiclox, erythromycin and amoxicillin.

Table 3: Antibiotic Susceptibility Pattern of *Streptococcus pneumoniae* isolates to various antibiotics used in the study

Isolates	No of Antibiotics to which Susceptible	Antibiotic Susceptibility Pattern	Multiple Antibiotic Resistance Index (M.A.R.I.)
1	4	CPX, S, PEF, GN	0.6
2	6	CPX, S, SXT, GN, Z, R	0.3
3	3	CPX,S, SXT	0.4
4	6	CPX, PEF, GN, APX, Z, R	0.3
5	4	CPX, S, PEF, GN	0.5
6	3	CPX, PEF, GN	0.7
7	5	CPX, S, PEF, GN, R	0.5
8	4	CPX, PEF, GN, R	0.5
9	4	CPX, PEF, GN,R	0.6
10	7	CPX, S, SXT, E, PEF, R	0.3
11	7	CPX, S, SXT, E, PEF, GN, R	0.2

KEY: CPX = ciprofloxacin, PEF = pefloxacin, GN = gentamicin, APX = ampiclox, R = ceftriaxome, S = streptomycin, SXT = co-trimoxazole, E =erythromycin, Z =cefuroxime.

Table,3 above highlights the number of antibiotics that each of the strain of *Streptococcus pneumoniae* isolate was susceptible to, their antibiotic susceptibility pattern and the multiple antibiotic resistance index (MARI). All the strains were susceptible to ciprofloxacin. Other antibiotics that were found active against most of the isolates include gentamicin, pefloxacin and streptomycin. The multiple antibiotic resistance indexes are high in each of the strains of *Streptococcus pneumoniae*.

DISCUSSION

The results arising from the findings of this study reflects that, of the sites of infection considered, Breast and the Skin has five (5) isolates of *Streptococcus pneumoniae* each, representing 45.5% of the total number of isolates discovered in each case. The fact that *S. pneumoniae* is associated with other infection other than pneumonia including, acute sinuses, otitis media, cellulites, bacteremia, sepsis (Jaime et al., 1986) supports the above.*S.pneumoniae* have also been isolated in various cases of malignant ulcer; for instance, in a similar study conducted on epidemiology and etiological diagnosis of corneal ulceration, *S.pneumoniae* was found to be the commonest bacterial pathogen(Srinivian et al.,1997).A study on microbial keratitis in a south Indian eye hospital revealed *S.pneumoniae* as the predominant bacteria (Bharathi et al.,2007).*S.pneumoniae* have also been isolated from patients conjunctiva and cornea in a retrospective study at the ophthalmology department of the National University of Asunction in Paraguay(Laspina *et al.,*2004),and in another retrospective study of infectitious keratitis in children at a University hospital in Asuncion-paraguay (Maidana et al.,2005) Alpha-hemolytic Streptococci have been reported most often to cause delayed wound healing and infection (Madsen *et al.*, 1996). Alpha-hemolytic Streptococci have also been reported to have adverse effect on wound healing by producing destructive virulence factors.

Ciprofloxacin showed 100% efficacy against all the isolates *Streptococcus pneumoniae* in this study. Pefloxacin and gentamicin were also highly active against the isolates, while the isolates were only moderately susceptible to streptomycin. The pattern of susceptibility of all the strains of *S. pneumoniae* to all the antibiotics used indicates that ciprofloxacin, pefloxacin and gentamicin appears to be a persistent combination of antibiotics that the isolates were susceptible to. Manjula et al. (2005) had earlier reported the susceptibility of *S. pneumoniae* to ciprofloxacin and gentamicin, which corroborates the finding in this study. The isolates of *S. pneumoniae* however showed resistance to amoxacillin, ampiclox, erythromycin and cefuroxime which agrees with the result of this study. Contrary to the findings in this study, Yaman et al. (2005) reported that *S. pneumoniae* is susceptible to Erythromycin. The high multiple antibiotic resistance index (MARI) that reflected among the strains of *S. pneumoniae* indicates that *S. pneumoniae* often colonized ulcerated wounds which must have led to indiscriminate use of antibiotics against it, making *S. pneumoniae* to become resistant to most of the antibiotics used, thereby reflecting high MARI.

CONCLUSION

The antibiotics that are found to be active against *S. pneumoniae* in this study, such as ciprofloxacin, pefloxacin and gentamicin could serve to effectively manage infected malignant ulcers in the study area. Patients need to be educated on the importance of reporting cases of chronic wounds to the hospitals early enough to avoid deterioration of such wounds that could lead to malignant ulcer.

REFERENCES

Bharathi M.J., Ramakrishnan R., Meenakshi R., Padmavathy S., Shivakumar C. and Srinivasan M., 2007. Microbial Keratitis in South India: influence of risk factors,climate,and geographical variation. Ophthalmic Epidemiol,**14**(2): 61-69.

Bjornson G., Scheifele D. Binder F. Talling D. ,2000 Population-based incidence rate of invasive pneumococcal disease in children: Vancouver 1994-1998. CCDR 2000-26. **18**:149-51

CDC.**,** 1997. Prevention of Pneumococcal Disease: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR, **46** (RR08):1-24.

Cheesbrough M., 2002. District Laboratory Practice in Tropical Countries. Part 2, Cambridge University Press, Cambridge, UK: 157-234.

Cybulaka, J., Jeeljasewicz, E.Lund, and Munkaguard A., 1970. Prevalence of types of Diplococcus pneumoniae and their suscepbility to 30 antibiotics. Chemotherapy, **15**: 304 - 316.

Dagan R., 2000. Treatment of acute otitis media challenges in the era of antibiotics resistance. Vaccine 19 Suppl **1 :** S 9 - S 1 6 . P M I D 1 1 1 6 3 4 5 7 (http://www.ncbi.nlm.nih.gov/pubmed/11163457).

Hanasman D., and Bullen M.M., 1967. A resistant Pneumococcus. Lancet ,**11**: 264-265.

Jaime S.C., Mark A.K., David V.G. and Raul J.C., 1986. Laboratory exercise in Microbiology. West Publishing Company,Los Angeles.San Francisco:254.

Laspina F., Samudio M., Cibils D.T.C.N., Farina N. Sanabria R., KlaussV., Mino D., Eand Kaspar H., 2004. Epidemiological characteristics of microbiological results on patients with infectious corneal ulcers: a 13-year survey in paraguay. Graefes Arch Clin Exp Ophthalmol, **242**(3):204-9.

Madsen E.L, Thomas C.T., Wilson M.S., Sandoli R. S., and Bilotta S.E., 1996. In Situ dynamics of aromatic hydrocarbons and bacteria capable of AH metabolism in a coal tar waste-contaminated field site. Environ. Sci. and Technol., **30**(7): 2412-2416

Maidana E., Gonzalez R., Melo Junior L.A. and Souza L.B., 2005. Infectious keratitis in Children:an epidemiological and microbiological study in a university hospital in Asuncion-paraguay.Arq Bras Oftalmol,**68**(6): 828-32.

Mandell LA. Marrie T.J., Grossmann R.F., Chow A.W., Hyland R.H., 2000. The Canadian Community-Acquired Pneumonia Working Group. Canadian guidelines for the initial management of community-acquired pneumonia: An evidence-based update by the Canadian Infectious Diseases Society and the Canadian Thoracic Society. Clin Infect Dis., **31**:383-421.

Manjula, M., Priya, D. and Varsha, G., 2005. Antimicrobial Susceptibility pattern of blood isolates from a Teaching Hospital in North India. Japanese Journal of Infectious Diseases, **58**:174-176.

NCCLS., 2002. Performance Standards for Antimicrobial Disc Susceptibility Test. Approved Standard MR-AS. National Committee for Clinical Laboratory Standard Villanova, PA USA:112-113.

Ryan K.J. and Ray C.G. (editors), 2004. Sherris Medical Microbiology. McGraw Hill.

Srinivasan M., Gonzales C.A., George C., Cevallos V., Mascarenhas J.M., Asokan. B., Wilkins J., Smolin G.,Whitcher J.P., 1997. Epidemiology and aetiological diagnosis of corneal ulceration in Madurai,South India. Br J Ophthalmol,**81**(11): 965-71.

Yaman A. Kibar F., Gurker N., Buyuk O. and Dundar I.H., 2005. Antibiotics susceptibility pattern and serotypes of streptococcus Pneumoniae invasive and other infections at University teaching Hospital in Turkey. 15th European Congress of clinical microbiology and infectious diseases, Copenhagen/ Denmark.