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ISOLATION AND ANTIBIOTIC SENSITIVITY OF *KLEBSIELLA PNEUMONIAE* FROM SPUTUM SPECIMENS OF PATIENTS OF FEDERAL MEDICAL CENTRE (F.M.C), UMUAHIA, ABIA STATE, NIGERIA.

Obi, C. N*

Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267, Umuahia, Abia State, Nigeria.

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***Correspondence for Author**
Dr. Obi, C. N
Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267, Umuahia, Abia State, Nigeria.

ABSTRACT

Fifty sputum specimens collected from patients attending Federal Medical Centre (F. M. C.) Umuahia were analyzed for the presence of *Klebsiella pneumoniae*. The antibiotic sensitivity pattern of the isolates was determined using the disc diffusion method. Of the 50 specimens, only 12 (24%) produced growth. The antibiotic sensitivity test revealed that all the isolates were sensitive to Ciproflox, 9 isolates (75%) were sensitive to Gentamycin, 6 isolates (50%) were sensitive to Augmentin, 5 isolates (41%) were sensitive to Nalidic acid, 4 isolates (33.3%) were sensitive to Ampicilin, 3 isolates (25%) were sensitive to septrin while 2 isolates (16%) was sensitive to both Travid and Streptomycin. 1 isolate (8.33%) was resistant to Gentamycin and Augmentin, 2 isolates (16%) were sensitive to Nalidixic acid, Ampicillin and Septrin, and 3 of the isolates (25%) were resistant to Travid and Streptomycin. All the isolates were completely resistant to Ceporex and Reflacine. The antibiotic sensitivity pattern of the isolate revealed that all the isolates were sensitive to Ciproflox and completely resistant to Ceporex and Reflacine.

KEYWORDS: Antibiotics, *Klebsiella pneumonia*, patients, sputum, Umuahia.

INTRODUCTION

Klebesiella pneumoniae is an opportunistic pathogen that causes various illnesses such as diarrhoea, septicemia, urinary and respiratory tract infections (Podschum and Ullmann,

1988). It is found in the environment and in mammalian mucosal surfaces. *K. pneumoniae* is a fastidious organism present as a normal flora in the mouth, skin and intestine (Ryan and Ray, 2004). It is clinically the most important member of the *Klebsiella* genus of enterobacteriaceae. It is an important medical species and in recent years, *Klebsiella* has become important pathogen in nosocomial infections (Yigit *et al.,* 2001).

K. Pneumoniae is found in patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy, and rhinoscleroma (Nina, 2009). Humans are the primary reservoir for *K. pneumoniae,* although organisms can also be found in soil and water. *Klebsiella pneumoniae* from coast water can be a zoonotic pathogen of marine mammals (Akoachere *et al*., 2009, Jangs *et al.,* 2010, Castinel *et al.,* 2007). Carrier rates of *K. pneumoniae* in humans ranges from 1 – 6 percent in the nasopharynx and are rarely carried on the skin (Podschun and Ullmann, 1988). Carrier rates are markedly increased in hospitalized patients in who reported rates are 77% in the stool, 19% in the pharynx and 42% on the hands. The higher rates of colonization are primarily related to the use of antibiotics (Podschun and Ullmann, 1988; Pollack *et al.,* 1972, Asensio *et al.,* 2000).

Resistance of *Klebsiella* specie to the cephalosporins such as oxymino beta-lactams was first described in 1980 and since then, a linear increase in resistance has been recorded. Their resistance is by the production of extended spectrum beta lactamases (ESBLS), (Iroha *et al.,* 2009). Although *K. pneumoniae* does not present a well defined clinical syndrome, its diagnosis in a hyper endemic focus may be made reasonable degree of certainly by an experienced clinician.

MATERIALS AND METHODS

COLLECTION OF SPECIMENS

Fifty sputum specimens were collected with 25 from both male and female adults and middle aged patients of Federal Medical Centre (F.M.C.) Umuahia using sterile plastic universal specimen containers. The sterile plastic universal containers were packaged in ice cubes in a plastic cooler and were transported to the microbiology laboratory of Michael Okpara University of Agriculture, Umudike for analyses.

PREPARATION OF MCFARLAND STANDARD

The McFarland standard was used for visual comparison to standardize the inoculums size. 1% v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid

to 99ml of water, and it was mixed adequately. The 1% v/v solution of barium chloride was prepared by dissolving 3.6 of dehydrated barium chloride in 200ml of distilled water. Then 0.6ml of the barium chloride solution was added to 99.4ml of Suphuric acid (H_2SO_4) solution and it was mixed evenly. A small portion of turbid solution was transferred into a screw cap Bijou bottle of the same type used in preparing the control inoculum. The standard was kept in dark, at room temperature 20 – 88℃ (Cheesbrough, 2000).

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF BACTERIA FROM SPUTUM SPECIMENS.

Each sputum specimen was inoculated into MacConkey (Fluka, Biochemika, Germany) and CLED (Bevis, Bury, England) agars previously prepared. A sterile wire loop was used to take a loop of the sputum specimen and was inoculated into different agar plate by streaking on the surface of the agar plates previously dried in the incubator. This was incubated at 37℃ for 24 hours to obtain isolated colonies. A fresh MacConkey (Fluka, Biochemika, Germany) and CLED (Bevis, Bury, England) agar media was prepared and the isolates were subculture on them. This was done by flaming the wire loop after each streaking to obtain a pure culture from the profuse growth of the previous culture. A sterile wire loop was used to take 3-5 well isolated colonies and emulsified in 3-4ml of physiological saline. Then the turbidity of the suspension was adjusted visually to the 0.5 Mcfarland's standard.

After incubation, the cultured plates were examined and characterized based on the colour, size, shape and morphology of the colonies. Gram stain reaction and some biochemical test were carried out. Characterizations of isolates were based on the result of various biochemical test (Cheesbrough, 1984).

ANTIBIOTIC SENSITIVITY PROFILE

Dics agar diffusion method originally described by Bauer *et al.*, (1960) was used in determining the antibiotic sensitivity profile. Muller Hilton agar (Scharlau Chemie, Spain) plates were prepared and dried with the lid of the Petri-dish slightly raised in a 40℃ incubator for about 30 minutes. A sterile swab stick was dipped in the standardized inoculum in a Bijou bottle. Excess fluid in the swab stick was removed by pressing and rotating the swab at the side of the bottle. The swab was used to seed all over the surface of the Muller Hilton agar plate (Scharlau Chemie, Spain). The plate was left for about 30 minutes, and then the antibiotic disc (Abtek Biological LTD) was aseptically removed with a sterile forceps. Each

disc was tightly pressed down so as to ensure proper contact with agar surface and left for another 30 minutes. The plates were then inverted and incubated aerobically at 37°C for 12-18 hours. Each sample was replicated 2 times, and the mean values were recorded. Inoculated plates were examined for zone of inhibition around the disc after incubation for 12 -18 hours. A transparent ruler was used to measure the diameter of each zone of inhibition around the disc in millimeter.

DISCUSSION

The result of this work showed that of the 50 specimens of sputum collected from patients of Federal Medical Centre Umuahia, 12 gave growths of *Klebsiella pneumonia* which is 24% of the whole specimens while 38 (76%) did not produce any growth.

Antibiotic sensitivity screening was determined by the zones of inhibition using the disc diffusion method for sensitivity test. The diameters of the zones of inhibition produced by the 12 isolates were compared with interpretative reference ranges according to Cheesbrough (2000). For sensitivity to antibiotics, the isolates gave the following results: Ciproflox (100%), Gentamycin, 75% (9 of 12); Nalidixic acid 41.7% (5 of 12); Augmentin 50% (6 of 12); Ampicillin 33.3% (4 of 12); Septrin 25% (3 of 12), Streptomycin 16.7% (3 of 12), and Travid 16.7% (2 of 12) respectivley. The pathogen was resistant to the antibiotics as follows: Ceporex, Ciproflox and Reflacin 100% (12 of 12); Ampicillin 16.7% (2 of 12), Streptomycin and Travid 25% (3 of 12); Nalidixic acid and Septrin 25% (2 of 12); Gentamycin and Augmentin 8.33% (1 of 12) respectively. Intermediate resistance was found against Streptomycin, 33.3% (4 of 12); Travid, Nalidixic acid, Augmentin and Ampicillin, 25% (3 of 12) respectively; Septrin 16% (2 of 12) and Gentamycin 8.33% (1 of 12) (Figure 1).

The complete resistance found against Reflacin and Ceporex was probably because of development of multidrug resistance due to prolonged use of these drugs against the pathogen within and outside the hospitals. The pathogen was 100% sensitive to Ciproflox (Figure 1) probably due to the fact that Ciproflox is not commonly used even in the primary care settings to treat *Klebsiella pneumoniae* infections, so strains resistance to it has not emerged. They act by inhibiting bacteria DNA gyrase or topoisomerism II, probably by binding to the DNA gyrase complex (John *et al.*, 2000).

The work showed that the isolate had 75% sensitivity to Gentamycin (Figure 1). Hence fluoroquinolones are widely regarded as optimal for the treatment of *Klebsiella pneumonia*

infection in adults (John *et al.*, 2000; Mittman *et al.*, 2002). Augmentin, Nalidixic acid, Ampicillin and Septrin produced limited amount of sensitivity respectively (Figure 1) probably due to multi-drug resistance that may be acquired from drug-resistance plasmids and the frequent use of these antibiotics. According to Rang *et al.*, (1999), resistance in bacterial populations can be spread from person to person, from bacteria to bacteria by plasmids and from plasmids to plasmids by transposons.

In this study too, Streptomycin and Travid gave only 16.7% sensitivity respectively (Figure 1) which signified that the isolates were highly resistant to these antibiotics. This could be due to the production of enzymes by the isolates that inactivated the antibiotics or probably because the *Klebsiella pneumoniae* generated different types of hybrid plasmids which must have consisted of the specific virulence plasmids which carried a gene cassettes. Most of gene cassettes contain resistance genes responsible for resistance to these conventional antibiotics.

Iroha *et al.*, (2009), reviewed that *Klebsiella* species is resistant to the β-lactams such as Ampicillin (Figure 1) by the production of the enzyme carbapenemase which makes it resistance to the drug carbapenem. This resistance is also by the production of extended spectrum of beta lactamases (ESBLS) which are encoded by transferable conjugative plasmids which often encode resistance determinant to other classes of antibiotics.

Klebsiella pneumoniae was isolated from 8 sputum specimens produced by males and 4 produced by females (Table 1). This indicates that the infection was found more in males than female patients sampled. This points to the fact that males are more prone to *Klebsiella pneumoniae*, probably because they take more of alcohol than the females which could also contribute or lead to liver problem as reviewed by Nina (2009).

The antibiotics sensitivity profile of this work showed that susceptibility patterns of bacterial isolates are constantly changing and as such the antibiogram of the bacteria isolates should be known before the initiation of treatment by a physician. Unfortunately, in the developing countries particularly Nigeria, physicians tend to prescribe antibiotics on predictions which often leads to treatment failure. Antibiotics sensitivity testing reveals the antibiotics of choice and hence, it is not useful but indispensable.

Efficacy, availability and cost are important criteria for the selection of first line antibiotics to be used in developing countries. Hence the choice of antibiotics, the dosage, regimen and the duration of therapy for adults may differ from those of children. The Fluoroquinolones are widely used for the treatemetn of *Klebsiella pneumoniae* in adults (John *et al.*, 2002, Prescott *et al*, 2006, Mittmann *et al.*, 2002). They are relatively inexpressive, well tolerated and more rapidly and reliably effective.

CONCLUSION

Indiscriminate use of antibiotics has resulted to the emergence and survival of resistance strains of bacteria isolates, thus antibiotic sensitivity is crucial for determining antibiotics of choice. Ciproflox and Gentamycin as revealed by this work were effective against most of the bacterial isolates and would do well if used in therapy.

Indiscriminate use of antibiotics in and outside the hospitals should be restricted and *Klebsiella pneumoniae* infection should be confirmed in a reference laboratory to avoid misidentification which could also lead to failure in treatment by administering the wrong antibiotics.

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