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Heavy Metal Resistance among *Kelbsiella* Isolates in Some Parts of Southwest, Nigeria

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Abstract: In this study, the heavy metal resistance among *Klebsiella* isolates in some parts of SouthWest Nigeria was investigated. A total of nine hundred and seventy (970) clinical specimens were collected out of which 544 isolates were recovered. The specimens were collected from four different states namely; Ekiti, Lagos, Ondo, and Osun state. Comparing the percentage relative distribution, Lagos state had 72.5% which was the highest while the lowest came from Osun state with 43.6%. Forty (40) isolates randomly selected from the states were screened for metal resistance using pour plate method. The metals used were copper, lead, zinc, magnesium, mercury and nickel. Resistance was 100% in lead and zinc, and also high (87.5%) with mercury and nickel (80.0%). Only magnesium (50.0%) and iron (40.0%) had resistance at lower ranges. This work suggested that the presence of these metals at this concentration (25 mg/mL) in hospital environments would not have significant effect on the occurrence of *Klebsiella*.

Key words: Clinical specimens, heavy metals, hospital environment, *Klebsiella*, resistance

INTRODUCTION

The genus *Klebsiella* belongs to the tribe *Klebsiellae*, in the family Enterobacteriaceae and was named after Edwin Kleb (1834 - 1913), a 19th century German microbiologist (Umeh, 2002). They have the general features of the other members of Enterobacteriacae but are not motile; they are capsulated both in the natural environment and as human pathogens (Podschun and Ullamann, 1994). Members of this genus *Klebsiella* typically express two types of antigens on their cell surface namely: K-antigens which is a lipopolysaccharide with 77 varieties and O-antigen, a capsular polysaccharide with varieties (Podschun and Ullmann, 1998). Their presence colonize the mucus membranes of mammals. In human, they are found in the epithelia of the nose and pharynx as well as in the intestinal tract. Nosocomial *Klebsiella* infections are caused mainly by the medically most important species, *Klebsiella pneumoniae.*

On the other hand, heavy metals are toxic chemical elements and their derivative chemical compounds. These heavy metals are frequently generating strong Reaction Oxygen Species (ROS) and directly or indirectly causing gene mutations and therefore their presence are hazardous to cells (Turer and Maynard, 2003). However, some of these heavy metals are necessary for life, namely: Copper, Iron and Zinc. These metals are essential trace elements that are required for a number of enzyme activities (Jarup, 2003). At a certain concentration level, these elements participate in some enzyme activities and when in excess, the toxic effects of these dual functional ions are revealed. In order to survive the wild, bacteria need to develop different mechanisms to confer resistance to these heavy and other metals (Karamanis *et al*., 2008).

There have been numerous studies on heavy metal resistance of bacteria isolated from different habitats (Jansen *et al*., 1994; Shi *et al*., 2002; Wakida *et al*., 2008). Therefore, this work was also focused to determine the heavy metal resistance among *Klebsiella* isolates in SouthWest, Nigeria.

MATERIALS AND METHODS

Sources of samples: Samples were collected from five different hospitals in four SouthWestern States, Nigeria for the isolation of *Klebsiella*: They included Ekiti (University Teaching Hospital, Ado-Ekiti and Federal Medical Centre, Ido-Ekiti), Lagos (State General Hospital, Broad Street, Lagos), Ondo (Federal Medical Centre, Owo) and Osun (Obafemi Awolowo University Teaching Hospital Annex, Ilesha). The study was carried out at the University of Ado-Ekiti, Ado-Ekiti Microbiology Laboratory.

Collection of samples: Samples collected include Urine, high vaginal swab, blood, ear swab, sputum, pus,

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Table 1: Distribution of *Klebsiella* species from different clinical sources

Clinical sources	*Klebsiella pneumoniae*	*Kl. Ozaenae*	*Kl. Rhinoscleromatis*	Total
Urine	106	-	34	140
Blood	10	8	-	18
Sputum	38	20	15	73
Ear swab	18	7	-	25
CSF	22	8	5	35
Semen	25	12	10	47
Nasal swab	34	15	12	61
Stool	29	15	10	54
High vaginal swab	38	30	23	91

Table 2: Result of resistance of *Klebsiella* isolates to metals

Metals (25 mg/mL)	Growth (mm)
Copper (Cu)	15(37.5)
Lead (Pb)	0(0.0)
Zinc (Zn)	0(0.0)
Magnessium (Mg)	20(50.0)
Iron (Fe)	24(60.0)
Mercury (Hg)	5(12.5)
Nickel (Ni)	8(20.0)

Values in Parentheses represent percentage growth; n = 40

cerebrospinal fluid, semen, stool and nasal swab. A total of 970 samples were examined for the presence of *Klebsiella*.

Isolation and characterization of the organism: It was carried out as described by Olutiola *et al*. (2000) and Fawole and Oso (2001). All the swabs samples were cultured directly on MacConkey agar (Oxoid) and incubated overnight at 37ºC. Bacterial colonies with characteristic mucous and pinkish colours were presumptively identified as *Klebsiella* spp. Further confirmations were done by carrying out certain biochemical tests.

Determination of susceptibility to metals: The effects of metals on forty (40) randomly selected representative organisms from four States were determined using pour plate method as described by Lane and Morel (2000). Seven (7) different metals namely: Copper (Cu), Lead (Pb), Zinc (Zn), Magnesium (Mg), Iron (Fe), Mercury (Hg) and Nickel (Ni) were selected for these tests.

A 1 mL aliquot of nutrient broth which contained the test organisms was placed in a clean sterile Petri dish. A prepared sterile nutrient agar medium which had been allowed to cool to about 45ºC was mixed with the inoculum in the Petri-dish and allowed to solidify. The discs which had been impregnated in a metal solution (25 mg/mL) was placed on each plate with the aid of sterile forcep and incubated at 37ºC for 24 h. Subsequently, the plates were observed for clear zones of inhibitions around the disc and the zones of inhibition were measured in millimeters.

RESULTS AND DISCUSSION

The results of the distribution of *Klebsiella* spp recovered from three different species *Klebsiella pneumoniae, Klebsiella ozaenae* and *Kl. rhinoscleromatis* from the nine different clinical samples in the selected States are as shown in Table 1.

It shows that out of the 544(56.1%) isolates of three species of *Klebsiella* recovered *Klebsiella pneumoniae* was highest in Urine 106(19.5%) followed by *Kl. rhinoscleromatis* 34(6.3%). For Blood, *Klebsiella pneumoniae* was 10(1.8%), while *Klebsiella ozaenae* was 8(1.5%). Sputum specimen had 38(7.0%) of *Klebsiella pneumoniae*, 20(3.7%) of *Klebsiella ozaenae*, while *Kl. rhinoscleromatis* was 15(2.8%). Ear swab had 18(3.3%) of *Klebsiella pneumoniae* and 7(1.3%) of *Klebsiella ozaenae*. Cerebospinal fluid (CSF) contained the three species with *Klebsiella pneumoniae* having 22(4.0%), *Klebsiella ozaenae* 8(1.5%) and *Kl. rhinoscleromatis* 5(1.0%). Semen had 25(4.6%) of *Klebsiella pneumoniae*, 12(2.2%) of *Klebsiella ozaenae* and 10(1.8%) of *Kl. rhinoscleromatis*. Nasal swab had 34(6.0%) of *Klebsiella pneumoniae*, 15(2.8%) of *Klebsiella ozaenae* and 12(2.2%) of *Kl. rhinoscleromatis*. Stool contained 29(5.3%) of *Klebsiella pneumoniae*, 15(2.8%) of *Klebsiella ozaenae* and 10(1.8%) of *Kl. rhinoscleromatis*. While High viginal swab had 38(7.0%) of *Klebsiella pneumoniae*, 30(5.5%) of *Klebsiella ozaenae* and 23(4.2%) of *Kl. rhinoscleromatis*.

The results of the resistance of the *Klebsiella* isolates to heavy metals (25 mg/mL) are shown in Table 2. Cu, Pb, Zn, Mg, Hg and Ni that were used gave interesting results. Resistance was 100% in case of lead and zinc and also high (87.5%) with Mercury and Nickel (80.0%). Only Mg (50.0%) and Fe (40.0%) had resistance at lower ranges. The result of this work agreed with the findings of Karbasizaed *et al*. (2003) who reported bacterial resistance to cadmium at 200 mg/mL Mercury (54.3 mg/mL) and Copper (1750 mg/mL) among coliforms isolated from nosocomial infections in a hospital in Isfahan, Iran. Similarly, it agreed with the findings of Kamala-Kannan and Lee (2008) who reported that *Bacillus subtilis, Paenibacillus favisponis, B. cereus, B. amyloliquefaciens* and *B. licheniformis* were all resistant to Manganese at 40 μg/mL but in contrast had lower resistance to mercury at 10 μg/mL.

In this study, lower ranges of resistance were recorded against Mg (50.0%) and Iron (40.0%). This could be attributed to the degree of polymetallic pollution on the type of organic constituents and presence of negatively charged ions like chloride in the medium which may bind with the metal and alter the bioavailability and toxicity of metals. (Bezverbnaya *et al*., 2008). Generally, the resistance to heavy metals could be due to specificities of plasmid-mediated metal resistance. This is in agreement with the work of Karbasizaed *et al*. (2003) who reported that some enterobacteria isolated from nosocomial infections harboured a conjugative plasmid (56.4 Kb) encoding resistance to heavy metals. Similarly, Ghosh *et al*. (2000) reported on transferable plasmids encoding resistance to various heavy metals in *Salmonella abortus equi*. Karamanis *et al*. (2008) and Wakida *et al*. (2008) also reported efflux pumping misuse of antibiotics and enzymatic detoxification to be mechanisms of resistance against bacterial isolates.

CONCLUSION

Since most of the isolates were resistant to the metals used, therefore, the presence of these metals at this concentration (25 mg/mL) in hospital environment would not have significant effect on the occurrence of *Klebsiella*.

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REFERENCES

Bezverbnaya, I.P., L.S. Buzoleva and S. Khristoforova, 2008. Metal-resistant heterotrophic bacteria in coastal waters of Primorye. Russia J. Mar. Biol., 31: 73-77.

Fawole, M.O. and B.A. Oso, 2001. Laboratory Manual of Microbiology. Spectrum Books Ltd. Ibadan, pp: 127.

Ghosh, A., A. Sinah, P. Ramteke and V. Singh, 2000. Characterization of large plasmid encoding resistance to toxic heavy metals in Salmonella abortus equi. Biochem. Biophys. Res. Communi., 272: 6-11.

Jansen, E., M. Michels, M. Van-Til and P. Doelman, 1994. Effects of heavy metal in soil on microbial diversity and activity as shown by the sensitivity-resistance index, an ecologically relevant parameter. Bio. Fertil. Soils., 17: 177-184.

Jarup, L., 2003. Hazards of heavy metal contamination. Brazilian Med. Bullet., 68: 425-462.

Kamala-Kannan, S. and K.J. Lee, 2008. Metal to lerance and antibiotic resistance of *Bacillus* sp. isolated from sundown bay sediments, South Korea. Biotechnol., 7: 149-152.

Karamanis, D., K. Stamoulis, K. Ioannides and D. Patiris, 2008. Spatial and seasonal trends of natural radioactivity and heavy metals in river waters of Epirus, Macedonia and Thesscha. Desalination., 224: 250-260.

Karbasizaed, V., V. Badami and G. Emtiazi, 2003. Antimicrobial, heavy metal resistance and plasmid profile of coliforms isolated from nosocomial infections in a hospital in Isfahan, Iran. 2(10): 379-383.

Lane, T.E. and F.M. Morel, 2000. A biological function for cadmium in marine diatoms. Proceedings of Acad. Sci., USA. pp: 4627-4631.

Olutiola, P.O., O. Famurewa and H.G. Senntag 2000. An introduction to general microbiology, hygiene Institute per universital heideberg. Fed. Republic Germany, pp: 267.

Podschun, R. and U. Ullamann, 1994. Incidence of *Klebsiella planticola* among clinical Klebsiella isolates. Med. Microbiol. Letters, 3: 90-95.

Podschun, R. and U. Ullmann, 1998. Klebsiella spp. as nosocomial pathogens, epidemiology, typing methods and pathogenicity factors. Clini. Microbiol., 4: 586-603.

Shi, W., M. Bischoff, R. Turco and A. Konopka, 2002. Long-term effects of chromium and Lead upon the activity of soil microbial communities. Appl. Soil Ecol., 21: 169-177.

Turer, D. and J.B. Maynard, 2003. Heavy metal contamination in highway soils. Comparison of Corpus Christi, TX and Cincinnati, OH shows organic matter is key to mobility. Clean Technol. Environ. Policy, 4: 235-245.

Umeh, O., 2002. Klebsiella Infections. Nucleic Acids Res., 28(24): 4974-4986.

Wakida, F.T., D. Lara-Ruiz, J. Temores-Pena, J.G. Rodriguezventura, C. Diaz, and E. Garcia-Flores, 2008. Heavy metals in sediments of the Tecate River, Mexico. Environ. Geol., 54: 637-642.