



Prevalence of *Klebsiella Pnuemonia* and their In-Vitro Susceptibility Studies among Cattle Traders in Maiduguri Cattle Market, Borno State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was undertaken to assess the occurrence of *Klebsiella pneumoniae* and their in-vitro susceptibility among cattle traders, herdsmen and butchers in Maiduguri cattle market, Borno state, Nigeria. Two hundred and twelve sample (212) sputum samples were collected using wide mouth sterile universal container and transported immediately to Medical Micro-Biology laboratory department, Centre of Excellence, university of Maiduguri teaching hospital (U.M.T.H.) for Laboratory diagnosed. The Sputum samples were culture on MacConkey and Blood agar media and isolated then, identified using Biochemical test which include indole, citrate utilization and urease tests, but only 15 sputum samples were found infected or positive to *Klebsiella pneumoniae*. And

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their Antimicrobial susceptibility of the *Klebsiella pneumoniae* isolate by disc diffusion method shows that *Klebsiella pneumoniae* are susceptible to these Antimicrobial Drugs: Pefloxacin (93.3%), Tarivid (100%), Sparfloxacin (80%), Ciprofloxacin (93.3%) and on the other hand *Klebsiella pneumoniae* are resistance to these Antimicrobial drugs; Streptomycin (73.3%), Septrin (80%), Augumentin (73.3%), Gentamycin (66.7%), and Amoxacillin (60%). In conclusion, recommendation have been made on how to curtail the spread of infection caused by *Klebsiella* in the environment, homes, and between patients. Healthcare providers are advice to follow the specific infection-control precautions.

Keywords: *Klebsiella pneumoniae*; Antimicrobial susceptibility; cattle traders; cattle market; and Nigeria.

ABBREVIATION

U.M.T.H :University of Maiduguri Teaching Hospital
 CDC : Center for Disease Control
 CRKP :Carbapenem-resistant *Klebsiella pneumoniae*
 CRE :Carbapenem-resistant *Enterobacteriaceae*
 MIC : Minimal Inhibitory Concentrations
 ESBL : Extended-spectrum beta-lactamases

1. INTRODUCTION

Klebsiella pneumoniae is a Gram-negative, non-motile, lactose-fermenting, rod-shape organism which is able to grow either with or without free oxygen, deeming it a facultative anaerobic and measures 2 µm by 0.5 µm [1]. This organism is also surrounded by a capsule, which increases its virulence by acting as a physical barrier to evade the host's immune response. This capsule also protects the cell from desiccation [2]. *K. pneumoniae* is a home-grown microorganism in that it resides in the microbiota of humans. It can be found in the mouth, skin, and intestinal tract, where it initially does not cause disease it serve as normal flora of the body, *Klebsiella pneumoniae* can progress into severe bacterial infections (opportunistic pathogen) leading to pneumonia, bloodstream infections, wound infections, urinary tract infections, and meningitis. Patients who require equipment such as catheters or ventilators are at high risk for infections. Also, a patient administered a course of broad-spectrum antibiotic treatment is at an even high risk due to the disruption of the normal flora of the bacteria in the body, deeming it more susceptible to pathogens [3]. *Klebsiella* organisms are often resistant to multiple antibiotics. Current evidence implicates plasmids as the primary source of the resistance genes [4]. *Klebsiella* with the ability to produce extended-spectrum beta-lactamases (ESBL) is

resistant to many classes of antibiotics. The most frequent are resistance to aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and trimethoprim/sulfamethoxazole [5]. Infection with carbapenem-resistant *Enterobacteriaceae* (CRE) or carbapenemase –producing *Enterobacteriaceae* is emerging as an important challenge in health-care settings [6]. One of many CREs is carbapenem-resistant *Klebsiella pneumoniae* (CRKP). Over the past 10 years, a progressive increase in CRKP has been seen worldwide; however, this new emerging nosocomial pathogen is probably best known for an outbreak in Israel that began around 2006 within the healthcare system there [7]. In the USA, it was first described in North Carolina in 1996 [8]. Since then CRKP has been identified in 41 states [9]. And is recovered routinely in certain hospitals in New York and New Jersey it is now the most common CRE species encountered within the United States. CRKP is resistant to almost all available antimicrobial agents, and infections with CRKP have caused high rates of morbidity and mortality, in particular among persons with prolonged hospitalization and those critically ill and exposed to invasive devices (e.g., ventilators or central venous catheters). The concern is that carbapenem is often used as a drug of last resort when battling resistant bacterial strains. New slight mutations could result in infections for which healthcare professionals can do very little, if anything, to treat patients with resistant organisms. A number of mechanisms cause carbapenem resistance in the *Enterobacteriaceae*. These include hyperproduction of ampC beta-lactamase with an outer membrane porin mutation, CTX-M extended-spectrum beta-lactamase with a porin mutation or drug efflux, and carbapenemase production. The most important mechanism of resistance by CRKP is the production of a carbapenemase enzyme, blakpc. The gene that encodes the blakpc enzyme is carried on a mobile piece of genetic material (a transposon

the specific transposon involved is called Tn4401), which increases the risk for dissemination. CRE can be difficult to detect because some strains that harbor bla_{KPC} have minimal inhibitory concentrations (MICs) that are elevated but still within the susceptible range for carbapenems. Because these strains are susceptible to carbapenems, they are not identified as potential clinical or infection control risks using standard susceptibility testing guidelines. Patients with unrecognized CRKP colonization have been reservoirs for transmission during nosocomial outbreaks. The extent and prevalence of CRKP within the environment is currently unknown. The mortality rate is also unknown, but is suspected to be within a range of 12.5% to 44%. The likelihood of an epidemic or pandemic in the future remains uncertain. The Centers for Disease Control and Prevention released guidance for aggressive infection control to combat CRKP: Place all patients colonized or infected with carbapenemase-producing *Enterobacteriaceae* on contact precautions. Acute-care facilities are to establish a protocol, in conjunction with the guidelines of the Clinical and Laboratory Standards Institute to detect non susceptibility and carbapenemase production in *Enterobacteriaceae*, in particular *Klebsiella* spp. And *Escherichia coli*, and immediately alert epidemiology and infection-control staff members if identified. All acute-care facilities are to review microbiology records for the preceding 6–12 months to ensure that there have not been previously unrecognized CRE cases. If they do identify previously unrecognized cases, a point prevalence survey (a single round of active surveillance cultures) in units with patients at high risk (e.g., intensive-care units, units where previous cases have been identified, and units where many patients are exposed to broad-spectrum antimicrobials) is needed to identify any additional patients colonized with carbapenem-resistant or carbapenemase-producing *Klebsiella* spp. and *E. coli*. When a case of hospital-associated CRE is identified, facilities should conduct a round of active surveillance testing of patients with epidemiologic links to the CRE case (e.g., those patients in the same unit or patients having been cared for by the same health-care personnel [10]. One specific example of this containment policy could be seen in Israel in 2007 [11]. This policy had an intervention period from April, 2007 to May, 2008. A nationwide outbreak of CRE (which peaked in March, 2007 at 55.5 cases per 100,000 patient days) necessitated a nationwide

treatment plan. The intervention entailed physical separation of all CRE carriers and appointment of a task force to oversee efficacy of isolation by closely monitoring hospitals and intervening when necessary. After the treatment plan (measured in May, 2008), the number of cases per 100,000 patient days decreased to 11.7. The plan was effective because of strict hospital compliance, wherein each was required to keep detailed documentation of all CRE carriers. In fact, for each increase in compliance by 10%, incidence of cases per 100,000 patient days decreased by 0.6. Therefore, containment on a nationwide scale requires nationwide intervention. In the United States, the reasons the CDC is recommending the detection of carbapenem resistance or carbapenemase production only for *Klebsiella* spp. and *E. coli* are: this facilitates performing the test in the microbiology laboratory without the use of molecular methods, and these organisms represent the majority of CREs encountered in the United States. Effective sterilization and decontamination procedures are important to keep the infection rate of this antibiotic-resistant strain, CRKP, as low as possible.

2. MATERIALS AND METHODS

2.1 Research Design

Prospective studies of cattle traders, herdsman and butchers in Kasuwan shanu. The study involves obtaining the sensitivity and resistivity of *Klebsiella pneumonia* isolated.

2.2 Study Area

Maiduguri cattle market known as “Kasuwan Shanu” Maiduguri metropolitan council, Borno state, Nigeria.

2.3 Sample Study Population

A total of two hundred (212) subjects were enrolled in this study including cattle traders, herdsman, and butchers, that are doing their business daily activities in Maiduguri cattle market.

2.4 Method of Sputum Collection (Expectoration)

Clean sterile wide mouth container (universal container) will be given to each subject, Orientation will be given to the subject by making

at least three breaths, then force out a deep or strong cough so that sufficient sputum may be expectorated into the sterile universal container.

2.5 Sample Processing

All sputum specimens were transported immediately to the laboratory and processed within an hour of collection.

2.6 Culturing Procedure

The specimens on reaching the laboratory were cultured on Blood, MacConkey agar respectively. They were incubated aerobically for 24 hours at 37°C. the following day, the plates were examined macroscopically for colonial morphological characteristics and features [12].

2.7 Statistical Data Analysis

The data generated was analyzed using Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL USA) version 23. Prevalence, sensitivity and resistivity was determined. The data were presented in tables.

3. RESULTS

Two hundred and twelve (212) sputum sample from cattle traders, herdsmen and butchers in Maiduguri cattle market were examine for the occurrence of *Klebsiella pneumoniae* and there in-vitro susceptibility, the following result were obtained.

Table 1. shows the demographic information of the respondent, number of samples, percentage

and frequency during the collection of the sample in Maiduguri cattle market Borno state, Nigeria.

Table 2. shows the prevalence, percentage and the number of organism isolated from two hundred and twelve sputum sample (212), ninety three (93) micro-organisms were isolated and identified, these are *Klebsiella pneumoniae* 15 (16.1%), *Escherichia coli* 8 (8.6%), *Pseudomonas* 7 (7.5%), *Proteus vulgaris* 10 (10.6%), *Proteus aeruginosa* 13 (13.9%), *Vibro cholera* 12 (12.9%), *Salmonellae spp*s 10 (10.6%), *Yersinia enterocolitica* 4 (4.3%), and others coliforms 14 (15.0%).

Table 3. shows the distribution of sensitivity and resistivity (susceptibility test) of *Klebsiella pneumonia* to antibiotics by disc diffusion method.

In all the 15 *Klebsiella pneumoniae* isolated, 15(100) are sensitive and 0(0%) are resistance to Tarivid, out of 15 of the *Klebsiella pneumoniae* isolate 5(33.3%) are sensitive and 10(66.7%) are resistance to Gentamycin out of 15 of the *Klebsiella pneumoniae* isolate 14(93.3%) are sensitive and 1(6.7%) are resistance to Pefloxacin 7(46.7%) are sensitive and 8(53.3%) are resistance to Chloranphenicol 13(86.7%) are sensitive and 2(13.3%) are resistance to Sparfloxacin 14(93.3%) are sensitive and 1(6.7%) are resistance to Ciprofloxacin 4(26.7%) are sensitive and 11(73.3%) are resistance to Streptomycin 3(20%) are sensitive and 12(80%) are resistance to Septrin 4(26.7%) are sensitive to and 11(73.3%) are resistance to Augmentin and finally out of 15 of the *Klebsiella pneumoniae* isolate 6(60%) are sensitive and 9(60%) are resistance to Amoxacillin.

Table 1. Demographic information of the sample collected

S/No.	Demographic	Respondents	No. of sample collected	Frequency	Percentage (%)
1	Age group	15-20	26	12.3	12.3
		21-26	31	14.6	14.6
		27-32	36	16.9	16.9
		33-37	42	19.8	19.8
		38-42	48	17.9	17.9
		43-47	24	11.3	11.3
		48-52	9	4.2	4.2
		53-57	6	2.8	2.8
		Total	212	100.0	100.0
2	Sex	Male	202	95.3	95.3
		Female	10	4.7	4.7
		Total	212	100.0	100
3	Marital status	Single	40	18.9	18.9

S/No.	Demographic	Respondents	No. of sample collected	Frequency	Percentage (%)
	Married		172	81.1	81.1
	Separated		0	0	0
	Total		212	100.0	100.0

Table 2. Numbers of micro-organism isolate from 212 sputum sample collected in Maiduguri cattle market among cattle traders Maiduguri, Borno State, Nigeria

S/No.	Name of organism	No. Of organism	Frequency	Percentage (%)
1	<i>Klebsiellae pneumoniae</i>	15	16.1	16.1
2	<i>E.coli</i>	8	8.6	8.6
3	<i>Pseudomonas</i>	7	7.5	7.5
4	<i>Proteus vulgaris</i>	10	10.6	10.6
5	<i>Proteus aeruginosa</i>	13	13.9	13.9
6	<i>Vibr cholera</i>	12	12.9	12.9
8	<i>Salmonellae spp</i>	10	10.6	10.6
10	<i>Yersinia enterocolitica</i>	4	4.3	4.3
11	<i>Others coliform</i>	14	15.0	15.0
	Total	93	100	100

Table 3. Distribution of sensitivity and resistivity (susceptibility test) by disc diffusion method of the 15 of the *Klebsiella pneumoniae* isolate

Sample No.	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	%	%
Antibiotics	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	Sens	Resi
Gentamycin	-	-	-	+	-	+	-	-	+	-	-	-	-	+	+	33.3	66.7
Pefloxacin	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	93.3	6.7
Tarivid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	0.0
Chloromphenic al	-	+	-	-	+	+	+	-	-	+	-	+	-	-	+	46.7	53.3
Sparfloxacin	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	86.7	13.3
Ciprofloxacin	-	-	-	-	-	-	-	-	+	+	-	+	-	-	+	93.3	6.7
Streptomycin	-	-	-	-	-	-	-	-	+	+	-	+	-	-	+	26.7	73.3
Seprtin	-	-	-	-	-	+	+	-	-	-	+	-	-	-	-	20.0	80.0
Augmentin	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	26.7	73.3
Amoxacillins	+	+	+	-	-	-	-	-	+	-	+	-	+	-	-	40.0	60.0

Key: + = zone of inhibition, - = No Inhibition, Sens. = Sensitivity, Resis. = Resistance

4. DISCUSSION

Klebsiella pneumoniae is found as a normal flora of the mouth, skin, and intestines. It causes destructive changes to human and animal lungs the mode of transmission is person-person contact if aspirated (inhaled), specifically to the alveoli (in the lungs) resulting in bloody sputum, and can progress into severe bacterial infections (opportunistic pathogen) leading to pneumonia, bloodstream infections, wound infections, urinary tract infections, and sometime meningitis, especially in immunocompromised individuals such as sufferers of chronic diseases or alcoholics [1]. This study was done to determine the prevalence of *Klebsiella pneumoniae* and there in-vitro susceptibility, among cattle traders,

herdsmen and butchers whose are always in contact with animals in Maiduguri cattle market known as "Kasuwan Shanu" Maiduguri metropolitan council, Borno state, Nigeria.

The study revealed that the prevalence of *Klebsiella pneumoniae* is low with 16.1%. This is in agreement with the study done by Darsana which stated that the prevalence of *Klebsiella pneumoniae* in slaughter house in Bali is low [13], and also in agreement with a study done by Founou which stated that the prevalence is 11.2% [14]. The high prevalence of *Klebsiella pneumoniae* as seen in this study may be as a result of environmental factor which favors the growth of the organism.

And the in-vitro susceptibility of *Klebsiella pneumoniae* isolate by disc diffusion method in this study shows that most 15 of the *Klebsiella pneumoniae* isolate are sensitive to these antimicrobial drug: Pefloxacin (93.3%), Tarivid (100%), Sparfloxacin (80%), Ciprofloxacin (93.3%) and resistance to Streptomycin (73.3%), Septrin (80%), Augumentin (73.3%), Gentamycin (66.7%), and Amoxicillin (60%).

5. CONCLUSION

The study showed that the prevalence of *Klebsiella pneumoniae* among cattle trader, herdsman and butchers in Maiduguri is low. Also, the study showed that the in-vitro susceptibility of *Klebsiella pneumoniae* isolate by disc diffusion method is sensitive to almost all the antimicrobial drugs used in the study.

6. LIMITATION OF THE STUDY

- Collection of sputum samples from cattle traders, herdsman and butchers at Maiduguri cattle market is an uneasy task due to people socio-cultural view, individual differences and human behavior.
- The sputum sample may be nasty and messy, so asking a person for such sample is embarrassing in the group of people which basically depend on their educational background and awareness about such type of research particular.
- Time may be a major step back during this study period.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval to conduct the research was obtained from the Research Ethics committees of Borno State Ministry of Health on the 2nd June, 2020 and was approved on the 11th August, 2020 with the number MMHSZ/0324/III/. After explaining the purpose of the study, informed written consent to participate in the study was provided by all participants. Participation was entirely voluntary. Anonymity and confidentiality were guaranteed and maintained

CONSENT FOR PUBLICATION

Not applicable. The manuscript does not contain any individual personal data in any form (individual details, images, or videos).

AVAILABILITY OF DATA AND MATERIALS

The dataset used in this study are available from the corresponding author based on reasonable request.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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