Prevalence of Carbapenem resistant Klebsiella pneumoniae infections in a Nigerian Teaching Hospital.

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ABSTRACT

Background: The emergence of carbapenem resistant *Klebsiella pneumoniae* (CRKP) is of great concern because of the limited options for treatment. While carbapenem resistant *Klebsiella pneumoniae* is increasingly identified worldwide, limited data is available in Africa. This study was undertaken to determine the prevalence of CRKP in LUTH and to provide phenotypic characterisation of the resistance mechanisms.

Methods: A cross sectional study with 153 isolates of *Klebsiella pneumoniae* obtained from clinical cultures of inpatients identified using Microbact 12A and 12B. All isolates were subjected to antimicrobial susceptibility testing using the Modified Kirby Bauer method and interpreted according to the CLSI guidelines. The modified Hodge test was used to detect carbapenemase production.

Results: The prevalence of carbapenem resisant *Klebsiella pneumoniae* was 5.2% and carbapenamase producing *K. pneumoniae* was 2.6%. Most of the *K. pneumoniae* isolates had high rates of antibiotic resistance to Cefotaxime 78.4%, Ceftazidime 67.3%, Genticin 71.9%, Ciprofloxacin 59.5%, Cefepime 10.5% and Amikacin 13.7%.

Conclusion: There was a high rate of carbapenem resistance in *Klebsiella pneumoniae*. The *K. pneumoniae* isolates were also multi-drug resistant. There is need for antibiotic stewardship.

Keywords: Carbapenem resistance, *Kelbsiella pneumoniae*, prevalence

INTRODUCTION

Klebsiella pneumoniae is a common cause of healthcare associated infections. It readily develops resistance to multiple classes of antibiotics including production of extended spectrum beta lactamase (ESBL). Prevalence of ESBL producing *Klebsiella pneumoniae* infection has been reported worldwide. Due to the emergence of ESBL's, carbapenems have been the agents of choice for the management of multidrug resistant *Klebsiella pneumoniae* infections[1].

Carbapenems are reserved for use in serious and life threatening infections suspected or proven to be caused by bacteria resistant to other commonly used antimicrobials[2]. They are mostly used in the intensive care units (ICUs) for initial empiric therapy or as definitive therapy after susceptibility testing, for a variety of serious infections which include ventilator-associated pneumonia (VAP), severe sepsis, intra-abdominal infections and other life threatening infections[3].

In developed countries where carbapenems have been used for the past 2 decades, owing to the selective pressure on the organism, carbapenem resistant *Klebsiella pneumoniae* have emerged[4]. The emergence of carbapenem resistant *Klebsiella pneumoniae* is of great concern because of the limited options for treatment. Therefore, carbapenems may become ineffective for treating multi-drug resistant Gram negative bacterial infections leaving only few available therapeutic options[5]. The implications of CRKP will include prolonged hospital stay, increased morbidity, disability and mortality, increased cost of hospital care and possible spread of multi-drug resistant organisms in the hospital and community.

The mechanisms of resistance of *K. pneumoniae* to carbapenems include the production of specific carbapenem-hydrolysing beta-lactamases (carbapenemases) or modification of outer membrane permeability and upregulation of efflux systems[6]. The carbapenemases include members of Ambler's class A, class B (metallo-beta-lactamase) or the class D (OXA) beta-lactamases. Carbapenem resistance in *K. pneumoniae* can be mediated by class A beta-lactamases including the *Klebsiella pneumoniae* carbapenemase (KPC) and Guiana Extended Spectrum (GES),[7,8]. It may also be conferred by the metallo beta-lactamase which include the imipenemases (IMP), Verona imipenemases (VIM) and the New Delhi metallo beta-lactamase (NDM-1) or rarely by the OXA-type carbapenemases[7].

While carbapenem resistant *Klebsiella pneumoniae* is increasingly identified worldwide, limited data is available in Africa. Prevalence of carbapenem

resistant *Klebsiella penumoniae* in Uganda was 15.6% [9] while prevalence of 11.9 % among carbapenem resistant gram negative bacilli was reported in Kano, Nigeria [10]. This study was undertaken to determine the prevalence of carbapenem resistant *Klebsiella pneumoniae* in LUTH and to provide phenotypic characterisation of the resistance mechanisms.

Methods

We conducted a cross sectional study to determine the prevalence of carbapenem resistant *Klebsiella pneumoniae*. Subjects were selected from inpatients at the Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos, a 761-bed tertiary care teaching hospital with approximately 11,000 admissions and 10,000 clinical microbiology cultures processed annually. The subjects were in-patients with clinical culture specimens that grew *K. pneumoniae* during the period from 1st July, 2010 through 31st December 2010 identified from the microbiology laboratory. Specimen included blood culture, wound specimens (wound biopsy, aspirates, and swabs) urine, cerebrospinal fluid and sputum.

The study was approved by the Ethics and Research committee of LUTH. Informed consent was sought and obtained from participants.

Microbiological methods

Clinical cultures were processed according to routine microbiological procedures and *K. pneumoniae* isolates were identified using Microbact 12A and 12B (Oxoid Cambridge UK) according to manufacturer's instructions.

The antimicrobial susceptibility of isolates was detected using the modified Kirby-Bauer disc diffusion method and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guideline[11]. The turbidity of test and control organisms was adjusted to 0.5Mcfarland standard and the inoculum prepared by making a direct saline suspension of isolated colonies from an 18 to 24 hour agar plate [11].

The susceptibility of isolates was tested against 10 antibiotics namely amoxicillin-clavulanic acid (20/10µg), ceftazidime (30ug), cefotaxime (30ug), cefipime (30ug), imipenem (10ug), meropenem (10ug), ertapenem (10ug) gentamicin (10ug), amikacin (30ug), and ciprofloxacin (5ug) [Oxoid, Cambridge UK]. All antimicrobial discs were stored in the refrigerator at 4°C and allowed to equilibrate to room temperature before testing. Quality control was done using *E.coli* ATCC 25922.

Modified Hodge test was done to detect carbapenemase production as described by CLSI [11]. Briefly, A 0.5 McFarland standard suspension (using direct colony suspension method) of 1:10 dilution of *E. coli* ATCC 25922 in saline was made and streaked onto a Muller Hinton agar (MHA) plate (Oxoid) with a swab over the entire sterile agar surface. The plate was allowed to dry for 3 to 10 minutes and a meropenem disc was placed in the centre of the plate. The *K. pneumoniae* isolate was inoculated in a straight line out from the edge of the meropenem disc, at least 20-25 mm in length. This was incubated at 35°C in ambient air for 18 hours. Following incubation, the MHA plate was examined for enhanced growth around the test streak at the intersection of the streak and the zone of inhibition. An enhanced growth was considered positive, while no area of enhanced growth was considered negative. A clear area seen around the streak without any enhancement is considered not interpretable. Quality control was done using *K. pneumoniae* ATCC BAA-1705 as positive control and *K. pneumoniae* ATCC BAA-1706 as negative control.

Data analysis was done in SPSS version 20. Categorical variables were presented as proportions and percentages in a tabular form and *p* value of less than .05 was considered to be statistically significant.

Result

Klebsiella pneumoniae was isolated from the clinical specimens of 153 in-patients comprising 72 (47.1%) males and 81 (52.9%) females (table 1). These patients were adults and children including neonates. The age groups of the in-patients were divided into neonates with mean age of 7.1 ± 7.6 (days); paediatrics with a mean age of 23.6 ± 30.5 (months) and adults with mean age of 44.9 ±17.5 (years).

Thirty one (20.3%) of the patients were admitted on the surgical wards, 40 (26.1%) in the neonatal wards, 31 (20.3%) in the medical wards, 24 (15.7%) in the paediatric wards and 20 (13.1%) in the obstetrics and gynaecology wards. In all the patients, *Klebsiella pneumoniae* was isolated from blood in 66 (43.1%), urine in 49 (32%), wound specimens in 36 (23.5%) and cerebrospinal fluid in 2 (1.3%).

Carbapenem resistant *K. pneumoniae* (CRKP) was isolated from 8 of the 153 patients giving a prevalence of 5.2%. This included 7 that were resistant to meropenem and 4 each that were resistant to imipenem and ertapenem. Three CRKP isolates were resistant to all three carbapenems, 1 was resistant to imipenem meropenem, 3 were resistant to only meropenem and 1 was resistant to only ertapenem;

The results of susceptibility testing of *K. pneumoniae* to different antibiotics showed that 78.4% of the *K. pneumoniae* were resistant to cefotaxime, 71.9% to gentamicin, 67.3% to ceftazidime, 59.5% to ciprofloxacin, 10.5% to cefepime, 13.7% to amikacin, 4.6% to meropenem, 2.6% to imipenem and ertapenem (table 2).

All the 8 CRKP were resistant to both ceftazidime and cefotaxime. The resistance rate of CRKP to gentamicin was 87.5%, 75% to ciprofloxacin and 50% to cefepime. Of the 8 CRKP, 6 (75%) were susceptible to amikacin (see figure 1).

Only 4 (2.6%) *K. pneumoniae* produced carbapenemase by the modified Hodge test (MHT) and 50% of the CRKP were carbapenemase producers.

Table 1: Demographic characteristics of all patients.

Variables	CRKP (%) n=8	Non CRKP (%) n =145	Total (%) n = 153
Sex			
Male	3 (37.5)	69 (47.6)	72 (47.1)
Female	5 (62.5)	76 (52.4)	81 (52.9)
Wards			
Surgical	3 (37.5)	28 (19.3)	31 (20.3)
Medical	3 (37.5)	28 (19.3)	31 (20.3)
Paediatrics	0	24 (16.6)	24 (15.7)
Neonatal	1 (12.5)	39 (26.9)	40 (26.1)
Obstetrics & Gynaecology	1 (12.5)	19 (13.1)	20 (13.1)
Intensive care unit	0	7 (4.8)	7 (4.6)
Specimen			
Blood	2 (25)	64 (44.1)	66 (43.1)
Urine	4 (50)	45 (31)	49 (32)
Wound specimen	2 (25)	34 (23.5)	36 (23.5)
Cerebrospinal fluid	0	2 (1.4)	(1.3)

CRKP = carbapenem resistant *Klebsiella pneumoniae*

Table 2: Antibiotic susceptibility profile of all *Klebsiella pneumoniae* isolates N=153

	Resistant, n (%)	Intermediate, n (%)	Sensitive n (%)
Amikacin	21 (13.7)	11 (7.2)	121 (79.1)
Cefepime	16 (10.5)	43 (28.1)	94 (61.4)
Cefotaxime	120 (78.4)	2 (1.3)	31 (20.3)
Ceftazidime	103 (67.3)	14 (9.2)	36 (23.5)
Ciprofloxacin	91 (59.5)	13 (8.5)	49 (32)
Gentamicin	110 (71.9)	4 (2.6)	39 (25.5)
Ertapenem	4(2.6)	0	149 (97.3)
Imipenem	4 (2.6)	0	149 (97.4)
Meropenem	7 (4.6)	0	146 (95.4)

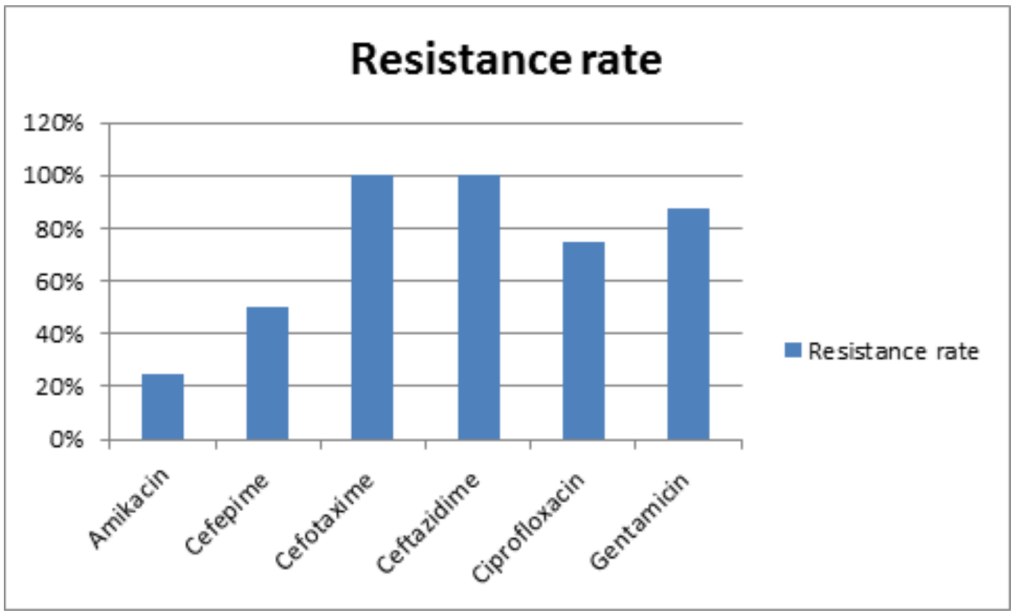

Figure 1: Antibiotic resistance rates of carbapenem-resistant *Klebsiella pneumoniae*

Discussion

This study shows that the prevalence of carbapenem resistant *Klebsiella pneumoniae* (CRKP) in patients admitted at the Lagos University Teaching Hospital during the period 1st July to 31st July 2010 was 5.2%. This prevalence rate of CRKP has increased at the same institution with study conducted in 2013 reporting 14.5% [12]. This increase may have been due to increased use of carbapenems in the hospital over the years. The prevalence rate of 5.2% is higher than a rate of 2.5% carbapenem resistant Enterobacteriaceae reported in Enugu Nigeria[13]. The prevalence rate in this study is still lower than the rate reported from Kano [10]. It was also lower than rates from other countries such as, 15.6% in Uganda [9], 12.9% in China[14], 46% in Greece[15] and 24% in New York, USA[16]. The higher prevalence in these countries has been attributed to consequence of failure to control the spread of CRKP. The prevalence rate of carbapenemase production was 2.6% by the Modified Hodge Test (MHT). However, the MHT only provides a high sensitivity and sensitivity for KPCs but does not easily detect metallo-beta-lactamases[17].

Carbapenemase production is associated with generalized resistance to carbapenems, penicillins, cephalosporins, aminoglycosides and quinolones[18]. All the CRKP isolates were multi-resistant with resistance to cefotaxime, ceftazidime, gentamicin and ciprofloxacin. Resistance to amikacin was significantly lower than the others but this antibiotic is less commonly used in LUTH. Despite the apparent sensitivity of CRKP to amikacin, studies have shown that amikacin did not demonstrate clinical success in the treatment of CRKP but was useful as part of combination therapy with carbapenems or colistin[19,20]. This resistance to multiple antibiotics is facilitated by the presence of genes encoding *Klebsiella pneumoniae* carbapenemase (KPC) on plasmids which also carry genes conferring extended spectrum beta-lactamases, aminoglycoside resistance and fluoroquinolone resistance[18].

The third generation cephalosporins are the most used antibiotics is this hospital accounting for 34% antibiotic prescriptions based on a point prevalence study[21]. In this study, only 20% of the isolates were susceptible to Cefotaxime which means that about 4 out of 5 patients prescribed this antibiotic will fail therapy. This rate of resistance is similar to that found in India[22]. This shows that third generation cephalosporins may not be a reliable empiric therapy for *Klebsiella pneumoniae* infections. Excessive use of third generation cephalosporins has been compounded by poor infection control practices especially hand washing which has aided the transmission of ESBL in the hospital. Hand washing practices in LUTH was poor as revealed in a study that showed only 11.7% health workers washed their hands before patient contact and 50.4 after patient contact[23].

The rates of resistance to other antibiotics were also high including ciprofloxacin at over 60%, gentamicin 75% and cefepime 30%. This shows that these isolates are multi-drug resistant. *K. pneumoniae* has been recognised during the past decade as a pathogen which very often is extensively drug resistant. Antibiotic resistance is largely driven by misuse and overuse of antibiotics. In Nigeria, antibiotics are sold over the counter without prescription by a medical doctor. This is worrisome in terms of the implications of antibiotic resistance in the hospital with increased treatment failure, cost of hospitalization, extended period of stay in the hospital and increased morbidity and mortality.

In conclusion, we found a high rate of carbapenem resistance in *Klebsiella pneumoniae*. The K. pneumoniae isolates were also multi-drug resistant. There is need for antibiotic stewardship to ensure appropriate use of antibiotics improvement in antimicrobial prescribing practices through formulation of good antibiotic guideline and policy. Strict infection control measures such as improving hand hygiene practices and instituting contact based isolation precautions should be implemented to prevent spread of CRKP.

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