Journal of Clinical Research Letters, ISSN: 0976–7061 & E-ISSN: 0976–707X
Vol. 1, Issue 2, 2010, PP-04-08

Prevalence and antibiogram of *Klebsiella* species recovered from clinical samples at the Komfo Anokye teaching hospital in Ghana

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Abstract-Background and Objective: The antibiogram of the various species of *Klebsiella* implicated in many infections in Ghana are not known. This study was designed to determine the antibiogram of the various species of *Klebsiella* isolated from clinical samples in Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana.
Materials and Methods: 2197 clinical specimens from the hospital were cultured on blood agar and MacConkey agar and the isolates investigated. The antibiogram of the various *Klebsiella* species isolated were determined and compared with antibiotics chosen for empirical treatment of 51 paediatric patients in the hospital.
Results: *Klebsiella* species were recovered from 205 clinical samples (9.3% prevalence rate) with *K. pneumoniae* being the highest recovered species (74.1%), followed by *K. oxytoca* (24.4%), *K. rhinoscleromatis* (1%) and *K. ozaenae* (0.5%). Amikacin was the most potent (80%) against the recovered species whilst cefotaxime, ceftriaxone and gentamicin exhibited approximately 60% potencies. Cefuroxime and ampicillin on the other hand were respectively 75% and 100% resistant.
Conclusion: The dominant species of *Klebsiella* infections were *K. pnuemoniae* and *K. oxytoca*. The third generation cephalosporins and aminoglycosides were the potent antibiotics for treatment of *Klebsiella* infections reported in KATH.
Key words: *Klebsiella* species, antibiotic resistance, aminoglycosides, cephalosporins.

Introduction

Klebsiella species are known to cause a variety of human infections such as pneumonia [1,2], septicaemia [3,4], urinary tract infections [5], rhinoscleroma, ozaena and other soft tissue infections [1,6]. *Klebsiella* are usually opportunistic pathogens found in the environment, on mammalian mucosal surfaces and on the hands of hospital personnel with the principal pathogenic reservoirs being the gastrointestinal tract of humans [1].

Different *Klebsiella* species may be responsible for different types of infections, and may also differ with the site of the infection. The various species of *Klebsiella* may exhibit different levels of susceptibility to antibiotics employed in treatment and also disinfectants used for cleaning purposes in the hospital. However, *Klebsiella* and many other pathogenic agents are rarely identified to species level in many hospitals in Ghana due to the cost and special skills involved. Hence antibiotics are administered to treat these infections without considering the species responsible for particular infection.

In this study, we seek to identify the species of *Klebsiella* implicated in infectious diseases in the Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana, and any variation in their antimicrobial susceptibility patterns. This will help in the selection of effective antibiotics for empirical treatment of *Klebsiella* infections at KATH as well as generating data on the prevalence of antibiotic resistant pathogens in Ghana and the sub-region.

Materials and methods

Cultivation and identification

The study was undertaken at KATH, Ghana, between May 2007 and March 2008. Different clinical samples of sputum, urine, blood, wound and ear swabs and aspirates (of pleural, gastric and knee fluids) collected from 2197 patients suspected of suffering from infectious diseases of the chest, ear, urinary tract were cultured to isolate the organisms. Demographic data (such as age, sex, in-patient and outpatient status) of the patients was recorded prior to sample collection.

There were no ethical matters concerned with this study, as results from routine laboratory diagnosis of clinical samples constituted the data for analysis; no particular identifiable group of patients were involved and their individual identities could not be traced. The samples were aseptically inoculated on plates of Blood and MacConkey agars (Oxoid Ltd, Basingstoke, UK) and incubated aerobically at 37^{o}C for 24 hours. The morphological characters of the colonies including sizes, shapes, colours, pigmentation and haemolytic nature and microscopic features of the cells were recorded. Suspected *Klebsiella* colonies were isolated and identified through biochemical tests according to Barrow and Felthan [7]. Citrate utilization, lysine decarboxylase and urease production as well as gas production during glucose fermentation differentiated *K. pneumonia* and *K. oxytoca* from *K. ozaenae* and *K. rhinoscleromatis* which were also MR positive and VP negative when

incubated at 37°C. Malonate utilization further differentiated *K. ozaenae* from *K. pneumonia, K. oxytoca* and *K. rhinoscleromatis* while indole production identified *K. oxytoca* from *K. pneumonia*. Cultivation of suspected *K. rhinoscleromatis* isolates on nutrient agar at 10°C for 24 h did not produce any observable colonies. *K. pneumonia* (NCTC 418. *K. oxytoca* (NCTC 5050 and *Enterobacter aerogenes* (NCTC 10006) were the reference strains employed.

Antibiotic sensitivity test

The Kirby-Bauer agar diffusion method of antimicrobial sensitivity testing [8] was employed to determine the antibiogram of the isolates. The inocula were prepared by growing *Klebsiella* species on Nutrient Agar (Oxoid, UK) plates for 18 h and the colonies formed were transferred into 3 ml of sterile normal saline in a test tube. The density of these suspensions was adjusted to 0.5 McFarland standards.

The surface of Muller-Hinton agar (Oxoid Cambridge, UK) plates were evenly inoculated with the organisms using a sterile glass rod. The antibiotic discs from Oxoid Cambridge, UK (ampicillin 10 µg, cefuroxime 30 µg, ceftriaxone 30 µg, cefotaxime 30 µg, gentamicin 10 µg, amikacin 30 µg, co-trimoxazole 25 µg, tetracycline 10 µg and chloramphenicol 10 µg) were placed on the surface of the agar using the antibiotic disc dispenser (Oxoid Cambridge, UK). The plates were then incubated at 37°C for 24 h and zones of growth-inhibition measured and compared to the chart supplied by National Committee for Clinical Laboratory Standards (NCCLS) [9]. Quality control was routinely performed using *Escherichia coli* ATCC 25922.

The empirical antibiotic treatments of 51 paediatric patients were also monitored and the data generated were compared with the sensitivity patterns of the isolates.

Results and discussion

Infectious diseases are the highest reported ailments encountered in many developing countries in Africa. The causative microorganisms of the diseases are increasingly becoming resistant to antibiotics employed in treatment hence the need to determine the antibiogram of these pathogens in order to evaluate the efficiency of empirical drug treatments formulated in our hospitals. In this study, *Klebsiella* species were recovered from 205 out of 2197 clinical samples collected in KATH (Table 1). Wound swab samples recorded the highest (31.2 %) recovered *Klebsiella* species followed by urine (26.3%), blood (20.0%), sputum (14.2%) and ear swab (4.4%). Pleural, gastric and knee aspirates also gave 2.4, 1.0 and 0.5 % recoveries respectively (Fig 1).

Prevalence of *K. pneumoniae* was the highest (74.1 %). The higher levels of recovery of this species from blood, wound swab, urine and sputum, supported the fact that it is the most virulent of all the *Klebsiella* [10] and hence the etiologic agent of both community and hospital acquired infections. *K. oxytoca* (24.4%) was the next highest species recovered from the samples except gastric and knee aspirates. *K. rhinoscleromatis* (1%) was also recovered from urine and wound swab whilst *K. ozaenae* (0.5%) occurred in only urine. These result compared favourably with those previously reported elsewhere [1,11]. Although *K. pneumoniae* is considered the commonest etiological agent of pneumonia among the *Klebsiellae*[2], it is worth noting that any of the *Klebsiella* species can produce infections in any anatomical site of the body when suitable conditions are available. The recovery of these other species in association with *K. pneumoniae* from the samples suggested their involvement in the progress of the infections suffered by these patients.

Among the two most prevalent species, *K. oxytoca* exhibited higher resistance to the antibiotics tested than *K. pneumoniae* (Fig 2). Both species were 100% resistant to ampicillin and between 75 – 94% resistant to tetracycline, co-trimoxazole, cefuroxime and chloramphenicol whilst gentamicin, ceftriaxone cefotaxime and amikacin were the least resisted (bellow 43%). These observations are consistent with those reported by Anderson *et al* [4], as well as Ohene [12] and Newman *et al* [8], in Ghana. These high antibiotic resistance levels are a predictable outcome of indiscriminate antibiotic use in many communities and hospitals [13]. Thus, health care facilities,

particularly the referral hospitals such as KATH with heavy antimicrobial use, could be the focal points in the emergence of antibiotic resistance [14,15]. Among the samples taken from empirically treated patients, 19 isolates out of the 51 were resistant to three or more of the antibiotics prescribed. This gave a multi-drug resistance prevalence rate of 37.3% and compares with the 37% obtained in Israel [16] but higher than the 22% recorded in Denmark [17].

The results of this study support the recommendation of the aminoglycosides (amikacin, gentamicin) and the third generation cephalosporins as suitable antibiotics for treating *Klebsiella* infections [18]. Gentamicin was the most prescribed empirical antibiotic followed by amikacin, cefotaxime and ceftriaxone (Table 2).

Though the third generation cephalosporins and amikacin are very potent, they are much more expensive than gentamicin [19]. Hence a little increment in the dosage of gentamicin may produce the same effect as amikacin [16] with minimal side effects and cost. This study has demonstrated that *Klebsiella species* are one of the commonest causes of infections among both out- and in-patients at KATH. Unless the antibiotic susceptibility test-result is known, ampicillin, tetracycline, chloramphenicol, co-trimoxazole and

cefuroxime should not be used for treatment of *Klebsiella* infection in Ghana.

Acknowledgement
We are grateful to Mr R. Lartey and the staff of the Clinical Microbiology Laboratory, KATH, for providing technical guidance and most of the logistics for this work.

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Table 1- Number of Klebsiella species recovered from clinical samples in KATH

Clinical Samples	No. of Clinical samples	*Klebsiella* Species Recovered				No. of *Klebsiella* positive Clinical Samples
		Kpn	Kox	Koz	Krh	
Blood	802	39	2	0	0	41
Ear swab	73	8	1	0	0	9
Gastric aspirate	31	2	0	0	0	2
Knee aspirate	22	1	0	0	0	1
Pleural aspirate	54	2	3	0	0	5
Sputum	206	27	2	0	0	29
Urine	502	33	19	1	1	54
Wound swab	507	40	23	0	1	64
Total	**2197**	**152**	**50**	**1**	**2**	**205**

Key: Kpn = *K. pneumoniae*, Kox = *K oxytoca*, Koz = *K. ozaenae* and K rh = *K. rhinoscleromatis*.

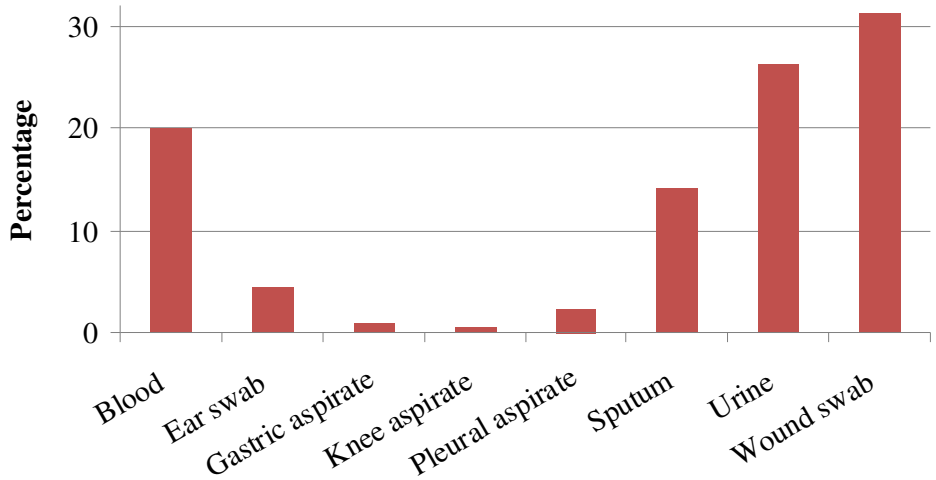

Fig 1. Prevalence of *Klebsiella* species amomg clinical samples in KATH

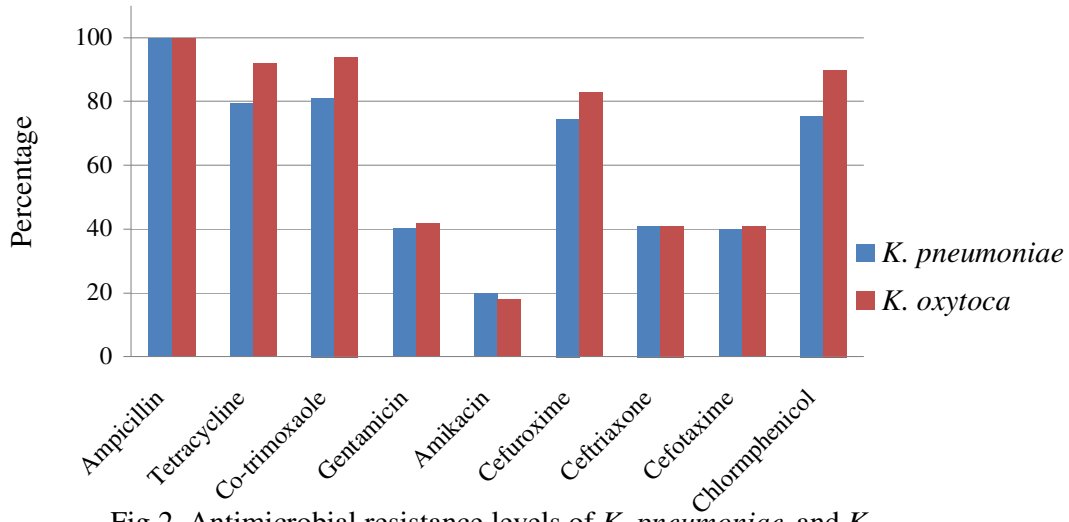

Fig 2. Antimicrobial resistance levels of *K. pneumoniae* and *K. oxytoca* in KATH

Table 2- Antibiotics employed in empirical treatment of Klebsiella infections in KATH

Antibiotics	Frequency of prescription	Percentage Frequency
Ampicillin	1	1.3
Tetracycline	2	2.7
Co-trimoxazole	4	5.3
Gentamycin	29	38.7
Amikacin	8	10.7
Cefuroxime	3	4
Ceftriaxone	6	8
Cefotaxime	7	9.3
Chloramphenicol	4	5.3
Ciprofloxacin	3	4
Floxacillin	2	2.7
Flucloxacillin	6	8
Total	75	100