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Occurrence and species distribution of *Klebsiella* Isolates: A case study at Komfo Anokye teaching hospital (Kath) in Ghana

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ABSTRACT

Different Klebsiella species may vary with the type of infections they cause in both the community and hospital environments. However, in many laboratories in developing countries, differentiation of the genus Klebsiella into species is not generally done during bacteriological diagnosis due to high cost and special skills involved. This study aimed at determining the prevalence of different Klebsiella species in KATH and how they relate to patients' demographic data. Two thousand one hundred and ninety seven (2197) clinical specimens from the hospital were cultured on blood agar and MacConkey agar and the isolates investigated. The isolates were identified to the species level using various biochemical tests. 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%). Occurrence of Klebsiella isolates in the commonest clinical specimens were sputum (14.1%), wound (12.6%), urine (10.7%) and blood (5.1%). The highest female cases were found in urine and the highest male cases were found in sputum. There was no significant difference between the out-patient and in-patient cases and among the sexes. The dominant species of Klebsiella infections were K. pnuemoniae and K. oxytoca. Pneumonia, UTI, Wound infections and Sepsis are the common infections of Klebsiella in Ghana. This study has also provided information for use in generating national data on the prevalence of pathogens in Ghana.

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. 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 Ghana, and how they relate to patients' demographic data.

MATERIALS AND METHODS

Isolation site

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.

Cultivation and Identification

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. pneumoniae* and *K. oxytoca* from *K. ozaenae* and *K. rhinoscleromatis* which were also MR positive and VP negative when incubated at 37^{o} 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^{o}C for 24 h did not produce any observable colonies. *K. pneumoniae* (NCTC 418. *K. oxytoca* (NCTC 5050 and *Enterobacter aerogenes* (NCTC 10006) were the reference strains employed.

Data analysis

The data from this study was subjected to statistical analysis using the methods as used by Koranteng-Addo et al. [12], Koranteng-Addo et al. [13], and Boamponsem et al. [14]. Significance differences among variables were tested at the 0.05 level. The software that was used for the data analysis was GENSTAT (Third edition).

RESULTS

Four *Klebsiella* species were recovered from 205 of the 2197 clinical samples collected and this gave a prevalence rate of 9.3 %. *Klebsiella pneumoniae* was the commonest (74.4%), followed by *Klebsiella oxytoca* (24.1%). There were 2 *Klebsiella rhinoscleromatis* isolates (1%) and *K. ozaenae* had only one isolate (0.5%) as indicated on (Table 1, and Figure 1).

Table 1. *Klebsiella* species isolated from various specimens in relation to in-patients and out-patients

		Species											
		K. pneumaniae			*K. oxytoca*			*K. ozaenae*			*K. rhinoscleromatis*		
Specimens Type	Number of Specimens	In-patient	Out-patient	Total	In-patient	Out-patient	Total	In-patient	Out-patient	Total	In-patient	Out-patient	Total
Blood sample	802	3	36	39	2	0	2	0	0	0	0	0	0
Ear swab	73	6	2	8	1	0	1	0	0	0	0	0	0
Gastric aspirate	31	2	0	2	0	0	0	0	0	0	0	0	0
Knee aspirate	22	1	0	1	0	0	0	0	0	0	0	0	0
Pleural aspirate	54	2	0	2	2	1	3	0	0	0	0	0	0
Sputum sample	206	11	16	27	0	2	2	0	0	0	0	0	0
Urine sample	502	23	10	33	10	9	19	0	1	1	0	1	1
Wound swab	507	13	27	40	10	13	23	0	0	0	0	1	1
Total	2197	61	91	152	25	25	50	0	1	1	0	2	2

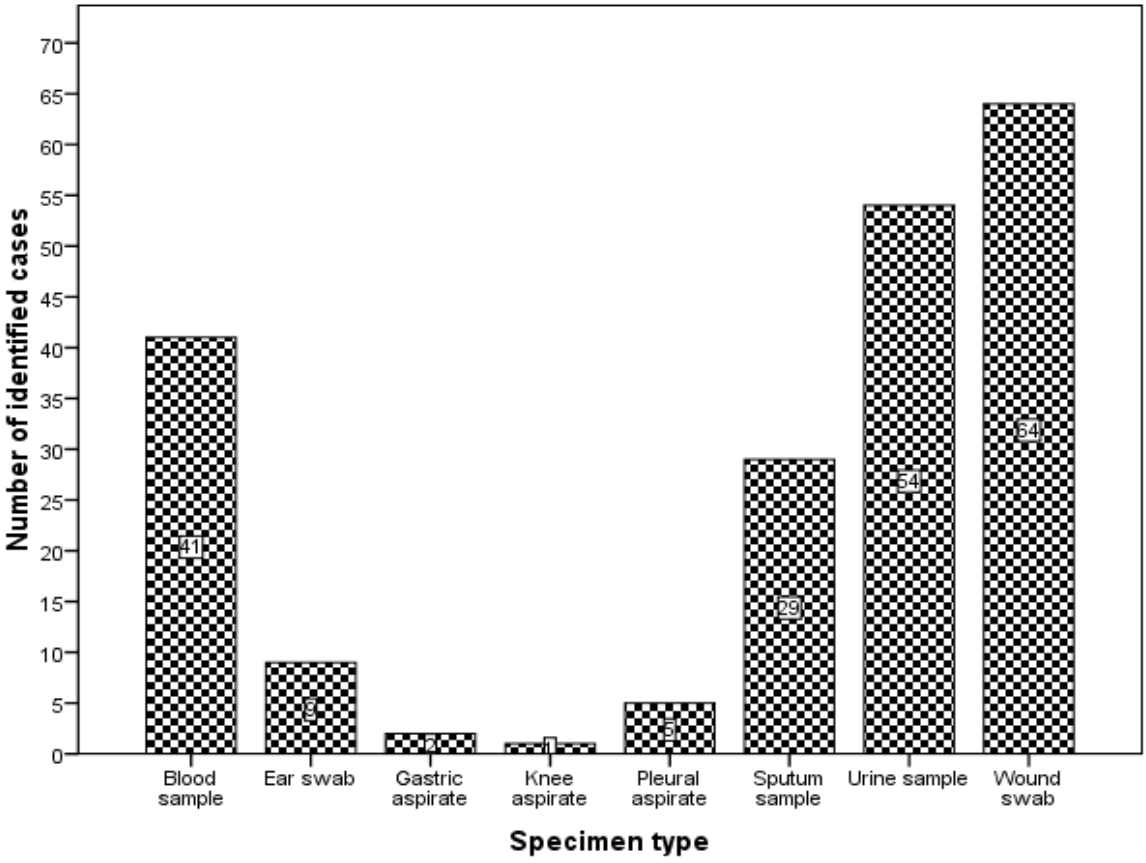

Figure 1. *Klebsiella* species isolated from examined samples

The isolates were obtained from 8 different specimens with the following percentage representations; wound (31.2%), urine (26.3%), blood (20%), sputum (14.1%), ear swab (4.4%), pleural aspirates (2.4%), gastric aspirate (1%) and knee aspirate (0.5%). *K. pneumoniae* was the commonest species isolated in all the specimens with the exception of pleural aspirate where it

had 2 isolates as against 3 of the *K. oxytoca.* Its highest isolates were from wound (40), blood (39), urine (33) and sputum (27). The highest isolates of *Klebsiella oxytoca* were from wound (23) followed by urine (19). The only *K. ozaenae* isolate encountered was from urine and the two isolates of *K. rhinoscleromatis* were recorded in urine and wound. Occurrence of *Klebsiella* isolates in the commonest clinical specimens was sputum (14.1%), wound (12.6%), urine (10.7%) and blood (5.1%).

There were a total of 103 out-patients and 102 in-patients. The in-patient cases were from seven wards in the hospital. Mothers babies unit (MBU) (21 cases), Block A (pregnant women) (19 cases), Block B (accommodate children) (16 cases), Pediatric emergency unit (PEU) (15 cases), Block C (accident unit) (14 cases), Block D (medical unit) (13 cases) and MEU (men emergency unit) (2 cases) as shown in Table 2 below. Out of the 152 *Klebsiella pneumoniae* isolated, 58.6% were from in-patients whereas 41.4% were from out-patients. *Klebsiella oxytoca* on the other hand had 50% for both in-patients and out-patients. Also, all the isolates of both *K. rhinoscleromatis* and *K. ozaenae* were from out-patients. The highest in-patient cases were from blood (90%) whereas ear swab (66.7%) and wound (64.1%) were the highest values for out-patients cases.

There were a total of 101 males and 104 females. As presented in Table 2, *Klebsiella pneumoniae* infection was detected among 74 females (48.7%) and 76 male (51.3%). *Klebsiella oxytoca* on the other hand was isolated from 27 females (54%) and 23 males (46%) of the isolates. The two isolates of *K. rhinoscleromatis* and the only *K. ozaenae* were isolated from females.

Table 2. Klebsiella species isolated in relation to the gender of patients

	Female		Male	
Species	Number	%	Number	%
K. pneumoniae	74	48.7	76	51.3
K.oxytoca	27	54	23	46
K. rhinoscleromatis	2	100	0	0
K.ozaenae	1	100	0	0

The ages of patients ranged between 1day to 85 years. The highest *Klebsiella* infection was detected in the age groups 1-9 and 30-39 years whereas age group 80-89 years was the least infected. The highest *K. pneumoniae* infection was detected in individuals in the age group 40-49 years (15.1%) whereas the highest *K. oxytoca* infection was found in the age group 30-39 years (18%).

Age group 10-19 years had the highest wound isolates (18.8%), age group 1-9 years had the highest urine isolates (20.4%), the highest blood isolates were from the population under one (<1) year (43.9%). The age groups 1-9 and 10-19 recorded the highest ear swab isolates (33.3%), the highest pleural isolates were from the age group 60-69 years (40%) whereas the highest sputum isolates were from the age group 40-49 years (40%).

DISCUSSION

Species identification is critical in the diagnosis and treatment of persons infected with *Klebsiella*. It is also required in disease prevention, patient management, and surveillance of infection. However, this practice is usually ignored in most of our hospitals mainly due to limited resources, time and labour. In this study we investigated the presence of *Klebsiella* species in 2197 clinical samples collected between May 2007 and March 2008 at KATH. Four *Klebsiella* species (*K. pneumoniae*, *K. oxytoca*, *K. ozaenae* and *K. rhinoscleromatis*) were identified to be responsible for causing infections in various anatomical sites. *K. pneumoniae* was the most common species isolated (74.1%), hence responsible for the majority of *Klebsiella* infections and therefore supported the fact that it is the most virulent of all the *Klebsiella* [8] and hence the commonest etiologic agent of both community and hospital acquired infections.

Prevalence of *Klebsiella* infections in the specimens encountered at KATH are 12.6% in wound swab, 10.7% in urine, 5.1% in blood, 14.1% in sputum, 12.3% in ear swab, 9.3% in pleural aspirate, 6.5% in gastric aspirate and 4.5% in knee aspirate. The prevalence rates recorded in this study are relatively higher than what was reported worldwide [1]. These relatively high figures may be due to the reason that, KATH is a teaching hospital and most of the cases which come here are usually referral cases from the hospitals in the northern sector of Ghana. Also it may be due to the fact that *Klebsiella* infections are gradually becoming common and difficult to manage in Ghana and for that matter in the sub-region.

There was a marginal difference betweem in-patients (102) and out-patients (103) cases recorded in the study, indicating that *Klebsiella* infections are common in both the community and the hospital. *Klebsiella pneumoniae* infections in in-patients (58.6%) were higher than in out-patients (41.4%), but a statistical analysis showed that the difference between them was not significant ($p < 0.005$). *Klebsiella oxytoca* on the other hand had equal cases for both in-patients and out-patients, whereas all the infections of *K. ozaenae and K. rhinoscleromatis* were out-patient cases. The highest out-patient cases were registered in ear swab and wound swab, therefore ear infection and wound infection may be considered as the commonest community-acquired *Klebsiella* infections in Ghana. Also the highest in-patient case was from blood. Septicemia (blood) may therefore be considered as the commonest hospital-acquired *Klebsiella* infection in Ghana.

Table 3. *Klebsiella* isolated in relation to sample type and ward

Wards	Blood	Urine	Wound	Sputum	Knee aspirate	Gastric aspirate	Ear swab	Pleural aspirate
Block A	2	10	1	1	1	2	0	2
Block B	1	4	11	1	0	0	1	0
Block C	0	6	4	3	0	0	1	0
Block D	2	0	5	4	0	0	1	1
PEU	10	3	2	0	0	0	0	0
MBU	21	0	0	0	0	0	0	0
MEU	0	1	0	0	0	0	0	1
OPD	5	30	41	20	0	0	6	1
Total	41	54	64	29	1	2	9	5

The Mother baby unit (MBU), Block A, Block B and Pediatric emergency unit (PEU) registered the highest *Klebsiella* infections (Table 3). These wards may therefore be considered as *Klebsiella* infection prone wards and may be sources of *Klebsiella* nosocomial infections. This conclusion was drawn from the figures recorded in this study and the fact that these wards have consistently being recording large numbers of *Klebsiella* infections according to the statistical records at the Microbiology Department of KATH.

The total number of females who were infected with *Klebsiella* was marginally higher than males. These figures show clearly that, *Klebsiella* infection is common among all sexes. This has been corroborated by other researchers [9].

The age groups 1-9 and 30-39 years were the most infected and may therefore be considered as the most prone to *Klebsiella* infections (Table 4). The least prone age group to *Klebsiella* infections was 80-89 years. The highest *K. pneumoniae* and *K. oxytoca* infections were detected in age groups 40-49 and 30-39 years respectively. The difference between infections of *K. pneumoniae* and infections of *K. oxytoca* in terms of age was highly significant (p>0.005).

Table 4. Age distribution among the clinical specimens encountered

Age group	W/S		Urine		Blood		E/S		P/A		G/A		K/A		Sputum	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
< 1	2	3.1	1	1.9	18	44	0	0	0	0	0	0	0	0	0	0
40552	6	9.4	11	20	11	27	3	33	0	0	1	50	0	0	0	0
40835	13	20.3	2	3.7	1	2.4	3	33	0	0	1	50	0	0	1	3.4
20-29	12	18.8	7	13	3	7.3	0	0	0	0	0	0	0	0	1	3.4
30-39	12	18.8	9	17	3	7.3	1	11	0	0	0	0	0	0	7	24
40-49	5	7.8	10	19	2	4.9	0	0	0	0	0	0	1	100	11	40
50-59	4	6.3	4	7.4	0	0	0	0	1	20	0	0	0	0	6	21
60-69	1	1.6	3	5.5	2	4.9	1	11	2	40	0	0	0	0	0	0
70-79	6	9.4	5	9.3	0	0	0	0	1	20	0	0	0	0	3	10
80-89	3	4.7	2	3.7	0	0	1	11	1	20	0	0	0	0	0	0

The highest urine isolates were registered by the age group 1-9 years and this may be due to the reason that UTI is very common among school age children especially girls [10]. Also the population under one (<1) year had the highest blood infections and this might be due to *Klebsiella* strains responsible for nosocomial infections since most of the patients were in-patients and babies who are usually immunocompromised and therefore susceptible to nosocomial infections and that, babies are usually prone to sepsis [10]. The highest wound isolates were from the age groups 10-19 years and since most of the patients were out-patients (64.1%) and the age groups in question are very active group and are usually involved in injurious activities, they are prone to injuries and easily exposed to *Klebsiella* infections. Also the highest sputum isolates were recorded by the age group 40-49 years and as reported, most cases of *Klebsiella* pneumonia occur in middle-aged and older men [11].

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