

PLASMID PROFILES OF KLEBSIELLA ISOLATES IN ILORIN, NIGERIA

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Antibiotic resistant organisms are most common in locations where antibiotics are in great use. This accounts for the fact that hospitals harbor many antibiotic resistant bacteria. It is not surprising that antibiotic-resistant organisms are more common in certain parts of the world, particularly in developing countries, which probably results from the over use of antibiotics. Many of this resistance in bacteria are mediated by plasmids. This study was carried out to identify factors responsible for poor clinical outcome in Klebsiella infections due to antibiotic resistance, and to detect the type of plasmids harbored by various strains of Klebsiella. Three hundred *Klebsiella spp.* were isolated from various clinical samples at the University of Ilorin Teaching Hospital and biochemically characterized. Five species were identified based on biochemical characteristics; *K. pneumoniae*, *K. rhinoscleromatis*, *K. ozaenae*, *K. planticola* and *K. oxytoca*. Plasmid was extracted and analyzed by Birnboim and Doly method. 55 (18.3%) had plasmids of different molecular weight with sizes ranging between 1.1 and 8.0 kb. Species that harbor plasmids are *K. pneumoniae* and *K. oxytoca*. It appears that plasmid is naturally occurring in some strains, but the incidence of plasmid is probably higher in areas where antibiotics are readily available to the general populace.

INTRODUCTION

Klebsiella is a genus of the family Enterobacteriaceae. Members of this genus are defined as Gram negative, non motile, aerobic to facultatively anaerobic bacilli which are catalase positive and oxidase negative (1). Enterobacteriaceae are a major component of the normal intestinal flora, but are relatively uncommon in other body sites (2). They are major cause of nosocomial infections, and may account for 80% of clinically significant isolates of Gram negative bacilli in clinical microbiology laboratories and 50% of all clinically significant isolates (3,4,5). Klebsiella are isolated in many types of human infections such as abscesses, pneumonia, meningitis, septicaemia, intestinal and urinary tract infections. Hospitalized patients often become colonized with *Klebsiella spp.* and among the Enterobacteriaceae, are a major cause of nosocomial infections (4). Nosocomial *K. pneumoniae* infection is associated with a high

mortality in both neonates and adults and antimicrobial treatments of the infection has been complicated by the emergence of multi-resistant strains (6).

Plasmids are circular extra chromosomal genetic elements that may encode a variety of supplementary genetic information including the information for self-transfer to other cells by conjugation and such properties as resistance to antibiotics (7). Plasmids carrying resistance factors are distributed through nearly all genera of medically important bacteria, with notable exceptions of *Neisseria meningitidis* and *Streptococcus pneumoniae* (8), and can be transferred via conjugation even between members of different species or genera (9). Multiply resistant *Klebsiella spp.* have been reported, and about 15-30% of Klebsiella are also resistant to broad-spectrum

cephalosporins by the production of R-plasmid encoded beta lactamases (10).

The present study was carried out to identify factors responsible for the poor clinical outcome in Klebsiella infections due to antibiotic resistance, and to detect the types of plasmids harboured by various strains of Klebsiella. We believe this can be used as an epidemiological tool in the control of infection during outbreaks caused by Klebsiella species within the hospital.

MATERIALS AND METHODS

All Klebsiella isolates from clinical specimens such as blood, urine, wound swabs pus, and aspirates between 1st January 2000 and 31st June 2000 were included in the study. Three hundred isolates of Klebsiella were examined by standard bacteriological techniques. All organisms provisionally identified as Klebsiella were first sub-cultured on to Blood and MacConkey agar plates and were incubated aerobically at 37°C. The organisms that conformed to the genus were further tested biochemically to differentiate them into species. The tests were; glucose fermentation for acid and gas production, lactose fermentation for acid production, dulcitol fermentation and glucose fermentation at 5°C. The other tests were Methyl Red (MR) and Voges Proskauer (VP) reaction (Table 1).

Plasmid was extracted using the modified Birnboim and Doly method (11). This is a mini scale isolation procedure based on the differential behaviour of closed circular, open circular and linear DNA under alkaline condition. The high alkalinity brings about the separation of the complimentary strands of DNA. Plasmid is then removed from the supernatant by ethanol precipitation. Electrophoresis of the extracted plasmid DNA

was carried out in 0.7-1% agarose gels in Tris borate buffer with a bromophenol blue tracking dye inside a horizontal slab gel apparatus. The gel was run for 4-5 h at 5v/cm constant voltage and then stained by immersing in water containing ethidium bromide (0.5 µg/ml) for 45 minutes at room temperature. The stained gel was visualized with a short wave ultra violet light transilluminator and the photograph of the plasmid bands on gel was taken using type 667 films on a Polaroid camera. *Escherichia coli* V517 was used as a control. This contained eight plasmid bands of different molecular weight. The relative mobility was calculated by measuring the distance of each band from the origin to the end point of electrophoresis.

RESULTS

Three hundred isolates were biochemically confirmed to be *Klebsiella spp.* Five species were identified based on biochemical characteristics; *Klebsiella pneumoniae*, 150 (50%), *K. oxytoca* 90 (30%), *K. rhinoscleromatis* 30 (10%), *K. planticola* 15 (5%) and *K. ozaenae* 15 (5%) (Table 2). Of the 300 isolates analyzed, 55 (18.33%) had plasmids of different molecular weights. The plasmid sizes ranged between 1.1 and 8.0 kilobases as shown in the plates. The calculation was done by finding the relative mobility of each gel (x), which was inputted into the equation; $Y = -2.15 X + 1.47$ where Y is the log of molecular weight of the plasmids. Species that harboured plasmids are *K. pneumoniae* 25 (45.6%), *K. oxytoca* 20 (36.4%), *K. planticola* 5 (9%) and *K. rhinoscleromatis* 5 (9%). Most of these strains were from urine and wound swabs. Multiple plasmids occurred mainly in *K. pneumoniae* as shown in the

plates. Plates 1 and 2 show the various plasmid bands (Lane 1-11). The control used was *E. coli* v517 with eight plasmid bands of different molecular weights.

Most of the isolates in plates 1 are *K. pneumoniae* with multiple plasmid bands, while the isolates in plate 2 belong to other species with single plasmid band.

TABLE 1: Biochemical characterization of Klebsiella species

KLEBSIELLA SPP	LAC	DUC	GAS IN GLU	GLU5°C	MR	VP
<i>Pneumoniae</i>	+	+	+	-	+	-
<i>Oxytoca</i>	+	+	+	-	-	+
<i>Ozaenae</i>	+		+	-	+	-
<i>Planticola</i>	+	+	+	+	-	+
<i>Rhinoscleromatis</i>	-	-	-	-	+	-

TABLE 2: Percentage distribution of Klebsiella species isolated

SPECIES	NUMBER OF ISOLATES	PERCENTAGE
<i>K.pneumoniae</i>	25	45.5%
<i>K. oxytoca</i>	20	36.4%
<i>K. planticola</i>	5	9.0%
<i>K. rhinoscleromati</i>	5	9.0%

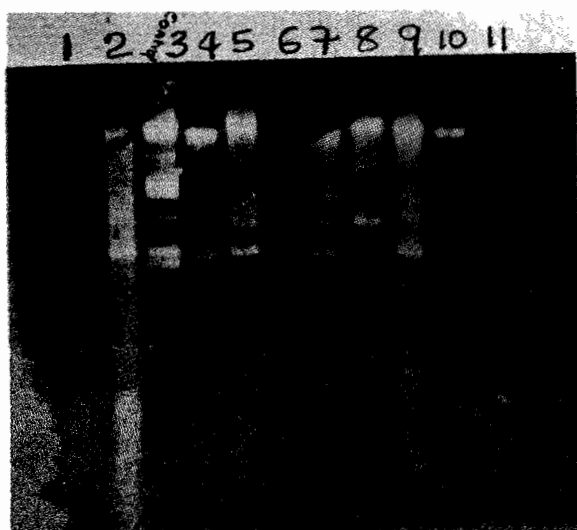


PLATE 1:- Plasmid fingerprints of *Klebsiella pneumoniae* lane 1 - 11 with *Escherichia coli* V 517 as control and having eight plasmid bands. Lanes 2, 4, 5, 7, 9 and 11 have plasmids of about 8 kilobases, Lanes 3, 6, 8, 10 and 11 have various plasmids lower than 8 kilobases.

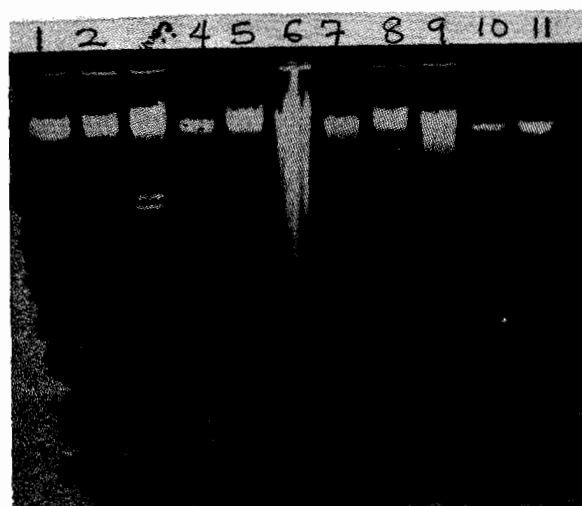


PLATE 2:- Plasmid fingerprints of *Klebsiella oxytoca*. Lane 1 - 11 with *E. coli* V 517 as control and having eight plasmid bands. Lane 2 has plasmid of about 8 kilobases, while lanes 3, 4, 5, 6, 7, 8, 9, 10, and 11 have plasmids lower than 8 kilobases.

DISCUSSION

Five species of *Klebsiella* were found in this study; *K. pneumoniae*, *K. oxytoca*, *K. rhinoscleromatis*, *K. planticola* and *K. ozaenae*. A similar study in Lagos in 1998 found only four species (12). It is noteworthy however that *K. pneumoniae* was found to be the predominant species in both studies, closely followed by *K. oxytoca*. In another study done in Lagos in 1985, over 90% of the clinical isolates of *Klebsiella* were *K. pneumoniae* (13) as against the 50% obtained in this study. From the above studies it can be observed that the most frequently isolated strains from clinical samples in Nigeria is *K. pneumoniae*, which is a recognized pathogen. It accounts for large number of hospital and community acquired infections involving the urinary tract, blood and lungs (14).

Fifty-five (18.55%) of the *Klebsiella* isolates harboured plasmids, majority of which were found in *K. pneumoniae* and *K. oxytoca*. Most of the plasmids were of low molecular weight ranging between 1.1 and 8.0 kb. Plasmids of lower molecular weight can usually be found in multiple copies in a single bacterium. In some studies done elsewhere, the presence of plasmids of lower molecular weight have been described as not related to bacteria resistance (15). Another factor that may be responsible in those without plasmids is the presence of β -lactamase production, which has been reported in *Klebsiella spp.* (16). Staphylococci often have resistance determinants distributed on several, small plasmids (17). Instability of these small plasmids apparently accounts for the variable antibiogram observed in isolates derived from a single colony of cultures obtained at different intervals from a single point.

From the plasmid fingerprints, some of the plasmids were shown to be related. Although multiple plasmids were observed in this study, the percentage was much lower than 50%. It is contrary to previous report (18) where a plasmid value of about 90% was isolated in some Gram negative clinical isolates of *Neisseria gonorrhoea*, *Campylobacter jejuni*, *Escherichia coli*, *Shigella dysenteriae* and *Salmonella spp.*, although *Klebsiella spp.* was not included in the study. It appears that plasmid occurs naturally in some strains of *E. coli*, but the incidence of plasmid is probably higher in countries where antibiotics are readily available to the general populace. A serious outbreak of *Klebsiella* infections in a neurosurgical unit was only brought under control when prophylactic ampicillin, which was routinely given to surgical patients, was stopped (19).

This study further highlights the need for proper antibiotic control. In the absence of antibiotic use or prescription, many of the bacteria strains spontaneously lose their plasmids. Antibiotics are probably overused for prophylactic purposes, and this has contributed to the development and spread of antibiotic resistance in bacteria strains.

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