Clinical sources and prevalence of resistance to antimicrobials of Klebsiella pneumoniae strains in Trinidad

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ABSTRACT

Objective: To assess the frequency of *Klebsiella pneumoniae* (*K. pneumoniae*) recovery from clinical specimens from hospital and community patients, their antibiotic profile, the extent of extended-spectrum beta lactamase (ESBL) production among such isolates, and the impact on patient management.

Methods: The study recovered 1,118 *K. pneumoniae* strains from various clinical specimens from hospital and community patients, from 1995 to 2004, at the Eric Williams Medical Sciences Complex. All 1,118 isolates underwent testing for multi-antibiotic resistance, of which 480 that fulfilled the criteria for possible ESBL production underwent further examination, according to guideline recommendations of the National Committee for Clinical Laboratory Standards.

Results: From 68,537 specimens processed, 62.9% were from hospital patients and 37.1% from community patients. Approximately 21% of the specimens yielded positive bacterial cultures from which, 1118 were *K. pneumoniae* strains. Ciprofloxacin, imipenem, aztreonam, nalidixic acid and gentamicin showed the greatest efficacy (>95% sensitivity) against isolates from both hospital and community sources. Tetracycline and ampicillin showed almost 100% resistances. The other antibiotics displayed varying degrees of resistance. The prevalence of ESBL production was approximately 8.5% and most ESBL producers (51.2%) were from urine, followed by wounds (22.0%), blood (19.5%), and lower respiratory tract specimens (4.9%). Five *K. pneumoniae* isolates were resistant to the carbapenem, and imipenem.

Conclusion: The study isolated *K. pneumoniae* from 8% of patients. All *K. pneumoniae* isolates were resistant to more than 2 antibiotics. The prevalence of ESBL production was 8.5%. Five (12.2%) strains of ESBL producers were resistant to imipenem. Continued infection control measures and prudent use of antibiotic agents are essential in reducing the spread of multi-resistant ESBL producing *K. pneumoniae*.

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Klebsiella pneumoniae is an important human pathogen associated with nosocomial and community-acquired infections worldwide.[1-3] We can commonly isolate these organisms from urinary tract infections,[4,5] eye infections,[6,7] pneumonias,[1,8,9] wound infections,[9,10] liver abscess,[11,12] and blood-stream infections.[2,9,13] Increasing resistance of *K. pneumoniae* to multiple antibiotics is therefore, a global problem,[1,14,15] and the extent of resistance to any particular antimicrobial agent varies with the therapeutic practice in that region, and among institutions which isolate *K. pneumoniae* strains. Extensive and often indiscriminate use of the late-generation (extended-spectrum) cephalosporins in particular, ceftazidime, cefotaxime and ceftriaxone, is associated with the emergence and spread of multidrug resistant *K. pneumoniae*.[1,15] The emergence of extended-spectrum ß-lactamase production (ESBL)

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among *K. pneumoniae* and *Escherichia coli* (*E. coli*), and their dissemination, greatly complicates therapeutic options for infections due to these organisms. These ESBL-producing isolates are resistant not only to the extended spectrum cephalosporins, but also to the aminopenicillins, ureidopenicillins, narrow-spectrum cephalosporins and aztreonam.[16] We can find resistance determinants encoding ESBLs on mobile genetic elements, facilitating their spread among members of the *Enterobacteriaceae* family, particularly *K. pneumoniae* and *E. coli*. Specific risk factors identified in patients colonized or infected with an ESBL-producing *K. pneumoniae* include admission to an intensive care unit (ICU), urethral catheterization, arterial catheterization, prolonged hospitalization, and prolonged exposure to antibiotics particularly the extended-spectrum cephalosporins.[17]

There are no reports on the clinical sources of Klebsiella organisms from any hospital in Trinidad, even though some reports included antimicrobial susceptibilities of such isolates.[9,10] Likewise, many resistant isolates escape ESBL-production detection with routine susceptibility testing and this can result in adverse therapeutic options. Antibiotic selection for treatment of infections due to ESBL-producers may create problems due to the complexity of in vitro susceptibility testing and in vivo correlation, and the widespread unawareness among clinicians of these organisms due to underreporting by the microbiology laboratory. The aim of the present study was to assess the frequency of *K. pneumoniae* as pathogens, the prevalence of ESBL-production and the extent of antimicrobial resistance among these organisms in an environment where over the counter antibiotics are readily available and the prevalence of resistance is high.

Methods. *Klebsiella pneumoniae* strains were isolated from various clinical sources in the Microbiology Laboratory of the Eric Williams Medical Sciences Complex (EWMSC), Trinidad, from January 1, 1995 through December 31, 2004 (**Table 1**). The EWMSC is a 560-bed semiprivate medical complex located in the northwestern part of Trinidad. Trinidad is the larger of a twin-island republic, Trinidad and Tobago, located approximately 11 kilometers off the northern coast of Venezuela in South America. The population of the Republic is about 1.3 million. Specimens were obtained from inpatients and outpatients. Inpatients are hospitalized patients, and outpatients are those attending the accident and emergency department, outpatient clinics, general practice and surrounding health centers. Only one isolate with a given resistant phenotype was retained for each patient for anti-biotic analysis.

Blood samples came in brain heart infusion broth (Oxoid, United Kingdom) and were incubated at 37^{0}C in a BACTEC (9050 series) machine until a positive signal (a beeping sound) was heard. Blood culture bottles with suspected bacterial growth were removed from the machine and observed for turbidity, gas or hemolysis before being subcultured onto blood, chocolate and MacConkey agar plates. Bottles with negative results were reported after the tenth day of incubation. Cerebrospinal fluid (CSF), urine ("clean catch" and catheter) and sputum specimens were collected in sterile containers. Specimens from wounds were collected onto sterile cotton Culturette II swabs (Becton Dickinson Microbiology System, Cockey-sville, Maryland, USA). All specimens collected with sterile cotton-tipped applicators (Culturette II), were inoculated onto sheep blood, chocolate and MacConkey agar plates and incubated aerobically at 35-37^{0}C for 18-24 hours. Urine samples were inoculated onto cysteine lactose electrolyte deficient and sheep blood agar plates using a platinum wire loop delivering 0.001 ml of urine. A midstream ('clean catch') urine specimen containing 100,000 bacteria per ml or >3,000 bacteria per ml in a catheter specimen of single species were considered as significant bacteriuria. Plates were incubated aerobically at 35-37^{0}C for 18-24 hours. All specimens were processed according to methods of standard procedures.[18] *Klebsiella pneumoniae* isolates were identified according to colonial morphology, Gram reaction and biochemical characteristics.

Susceptibility to various antimicrobials was carried out using the agar disc diffusion technique on Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Maryland, USA), according to guideline recommendations of the National Committee for Clinical Laboratory Standards (NCCLS).[19] Antibiotic discs containing the following concentration (in brackets) were used, ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), nalidixic acid (30 µg), gentamicin (10 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), cefuroxime (30 µg), ceftazidime (30 µg), ceftazidime-clavulanic acid (30/10 µg), cefotaxime (30 µg), cefotaxime-clavulanic acid (30/10 µg), imipenem (10 µg), aztreonam (30 µg) and ciprofloxacin (5 µg) (Becton Dickinson Microbiology System, Cockeysville, Maryland, USA). Antibiotic discs were obtained from several local drug distributors and not from a single supplier. Results were read the following day and interpretation of resistance, intermediate resistance and susceptibility was according to zone size range as recommended by the NCCLS.[19] Intermediate zones sizes were occasional and were excluded from the study.

Klebsiella pneumoniae isolates that were resistant to third generation cephalosporins and Aztreonam were tested for ESBL production. Following the inoculation of Mueller-Hinton plates, ESBL

presence was assayed using the following antibiotic discs (Becton Dickinson, Cockeysville, Maryland, USA): ceftazidime (30 μg) and ceftazidime-clavulanic acid (30/10 μg); cefotaxime (30 μg) and cefotaxime-clavulanic acid (30/10 μg), and interpreted according to NCCLS criteria.[19] The ESBL production was detected using the double–disc synergy (DDS) test.[20] The antibiotic and its combination discs were arranged in pairs so that the distance between them was approximately twice the radius of the inhibition zone produced by cefotaxime or ceftazidime tested on their own. Results for ESBL producers were reported as resistant to all penicillins and cephalosporins, and Aztreonam.[19] For all ESBL-production the control organisms were *E. coli* ATCC 25922 strain and *K. pneumoniae* ATCC 700603 strain obtained from the Caribbean Epidemiology Center, a branch of the Pan American Health Organization.

Results. The total number of specimens processed during the 9-year study period was 68,537, of which 43,131 (62.9%) were from hospital patients, and 25,406 (37.1%) from community patients. Of the 68,537, 20.7% (14,186) yielded positive cultures, from which 1,118 (8%) were common *K. pneumoniae* strains. Hospital patients accounted for 708 isolates (63.3%) and community patients, for 410 (36.7%) (**Table 1**). The major sources of *K. pneumoniae* from both hospital and community were urine and wounds, which together accounted for 67.8% (758) of all isolates. The resistance profile of *K. pneumoniae* strains isolated from both patient sources is shown in **Table 2**. Ciprofloxacin, imipenem, aztreonam, nalidixic acid, and gentamicin showed the greatest efficacy against isolates in both hospitalized and nonhospitalized patients showing >95% sensitivity. The antimicrobials with the lowest efficacy were tetracycline and ampicillin, with almost a 100% resistance to ampicillin in both practices, and approximately 90% resistance to tetracycline. All the other drugs displayed varying degrees of susceptibility. The prevalence of ESBL production is depicted in **Table 3**. Of the 480 *K. pneumoniae* strains tested, 35 (7.3%) were positive for ESBL production among hospital practices, and 6 (1.3%) and community practices. The ESBL-producing strains of *K. pneumoniae* was recovered from all specimen sources, with the majority (21 [51.2%]) from the urine of patients with urinary tract infections. Nine (22%) were from infected wounds, 8 (19.5%) from the blood of bacteremic patients and 2 (4.9%), from patients with lower respiratory tract infections. The remaining one (2.4%) ESBL-producing *K. pneumoniae* strains was from a high vaginal swab specimen.

Table 1 - Clinical sources of *Klebsiella pneumoniae* strains isolated at the Eric Williams Medical Science Complex 1995-2004.

Nature/source of specimen	Number of strains obtained (%)	
	Hospital	Community
Urine	345 (48.7)	188 (45.9)
Blood	116 (16.4)	0
Wound	110 (15.5)	115 (28)
Sputum	83 (11.7)	10 (2.4)
Abscess/pus	45 (6.4)	11 (2.7)
HVS	19 (2.7)	74 (18)
Miscellaneous*	5 (0.7)	12 (3)
Total	**708 (100)**	**410 (100)**

*peritoneal fluid; ear swab; umbilical swab; pleural fluid; eye discharge. HVS - high vaginal swab

Table 2 - Resistance profile of *Klebsiella pneumoniae* strains isolated from clinical sources at the Eric Williams Medical Science Complex 1995-2004.

Antimicrobial	Prevalence of resistance		
	Hospital (n=708)	Community (n=410)	Total (n=1,118)
	n (%)	n (%)	n (%)
Ampicillin	708 (100)	405 (98.8)	1,113 (99.6)
Tetracycline	639 (90.3)	377 (92)	1,016 (90.9)
ACA	273 (38.5)	127 (31)	400 (35.8)
Co-trimoxazole*	229 (32.3)	84 (20.5)	313 (28)
Cefuroxime	208 (29.4)	95 (23.2)	303 (27.1)
Ceftazidime	92 (13)	53 (12.9)	145 (13)
Gentamicin	43 (6)	10 (2.4)	53 (4.7)
Nalidixic acid	18 (2.5)	31 (7.6)	49 (4.4)
Aztreonam	10 (1.4)	11 (2.7)	21 (1.8)
Imipenem	5 (0.7)	0	5 (0.4)
Ciprofloxacin	2 (0.3)	10 (2.4)	12 (1.1)

*Trimethoprim-sulfamethoxazole,
ACA - Amoxicillin-clavulanic acid

Table 3 - Distribution of 480 *Klebsiella pneumoniae* strains from hospital and community sources according to extended-spectrum ß–lactamases (ESBL) production at the Eric Williams Medical Sciences Complex 1995 - 2004.

ESBL	Number of isolates tested (%)		
	Hospital	Community	Total
Producers	35 (10)	6 (4.7)	**41 (8.5)**
Non-producers	316 (90)	123 (95.3)	**439 (91.5)**
Total	**351 (100)**	**129 (100)**	**480 (100)**

Discussion. Klebsiella organisms cause a substantial degree of nosocomial infections.[21,22] Most nosocomial pathogens tend to acquire new antibiotic resistance which limit therapeutic options, thus, underscoring the need for updated antibiotic susceptibility patterns for the effective management of infections caused by these organisms.[15,22] The present study found that the frequency of isolation of *K. pneumoniae* at the EWMSC (8%) from among all bacterial isolates is comparable with that reported from Israel (12-13%),[23] but much lower than that reported from Ethiopia (15%)[24] and Oman (20%).[25] We recovered *K. pneumoniae* most frequently from infected urine and wounds. Urinary tract infection is the most common hospital-acquired infection, and it is therefore not surprising that *K. pneumoniae* strains are among the most frequently recovered etiologic agent from hospital and certain nonhospital sources.[26,27] This is probably the explanation for the high recovery rate of *K. pneumoniae* from urine in hospitalized patients (48.7%), where most patients receive urethral catheterization at some time during their hospital stay. Similarly, the high rate of isolation from community sources (45.9%) maybe explained in part, by patients with long-term urethral catheterization.[28] These patients are mostly men with outlet obstruction as a result of prostatic diseases. Catheters are left in situ (and changed monthly or earlier if there are complaints of fever, gross hematuria or acute blockage), as these patients are either awaiting surgery, are unfit for surgery or who refuse surgery.[28] Although we associate *K. pneumoniae* with eye infections and liver abscesses,[11,12] we did not recover from these sites in this study. We observed a high percentage of *K. pneumoniae* resistant to ampicillin and to tetracycline in this study. Most Klebsiella are naturally resistant to ampicillin and carbenicillin, but resistance to tetracycline is plasmid-mediated. Resistance to tetracycline reached a peak of 99% in 1995,[29] fell to 61.7% in 1997[9] and rose again to approximately 90% in this study. The reason for this is not entirely clear, but one possible explanation may be annual fluctuations in resistance probably due to logistics problems in getting antibiotics to this country from outside sources. Tetracycline is a relatively cheap drug, widely prescribed prophylactically to outpatients with chronic indwelling urethral catheters.[28] Resistance rates of cotrimoxazole, cefuroxime and ceftazidime remained relatively stable through out the study period, and resistance patterns of gentamicin (4.7%), nalidixic acid (4.4%), imipenem (0.4%) and ciprofloxacin (1.1%) were very low. The emergence of strains resistant to multiple antibiotics limits the therapeutic choices for nosocomial infections. Also, because *K. pneumoniae* is an important pathogen frequently responsible for nosocomial infections, strains producing ESBL are more prevalent and difficult to eradicate because they develop resistance to multiple antibiotics.[30]

Reports of treatment failure and nosocomial infections due to ESBL-producing organisms are emerging.[31,32] We recovered 29 (70.7%) of the ESBL *K. pneumoniae* identified in this study from hospital practice from patients admitted to the ICU. Patients admitted to our ICU usually have identifiable risk factors for acquiring ESBL-producing organisms such as *K. pneumoniae*. They include prolonged hospitalization, the use of invasive or diagnostic procedures and prior therapy with ß-lactam antibiotics such as the cephalosporins.[30,33] Identifiable risk factors for nonhospitalized patients include age over 60 years, male gender, diabetes, prior use of second generation cephalosporins and previous infection with *K. pneumoniae*.[34] One disturbing observation in this study was the 5 *K.pneumoniae* strains from hospitalized patients found to be resistant to imipenem. Two reports from the literature described emergence of an imipenem-resistant isolate of *K.pneumoniae* that produced a plasmid-mediated AmpC-type ß-lactamase accompanied by loss of outer membrane protein.[35,36] Many investigators recommend imipenem as the treatment of choice for serious infections caused by ESBL-producing organisms.[1,15,37] It is therefore, not surprising that imipenem is the drug we most commonly use to treat patients in outbreaks due to ESBL-producing organisms. In this light, increased use of imipenem in one study, resulted in a 70% increase in the prevalence of imipenem-resistant *Pseudomonas aeruginosa*.[38]

In conclusion, this study showed that <10% (41 of 1,118) of *K. pneumoniae* were ESBL producers. Despite this small percentage, the fact that 5 *K. pneumoniae* strains were resistant to imipenem is cause for serious concern. Microbiology laboratories should be aware of this fact and communicate the same to clinicians who will adjust therapy to more appropriate drugs; a fluoroquinolone such as ciprofloxacin is a good alternative.

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