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RESEARCH ARTICLE

ANALYSIS OF ANTIBIOTIC SUSCEPTIBILITY OF *KLEBSIELLA PNEUMONIAE* STRAINS ISOLATED FROM DIFFERENT CLINICAL SPECIMENS IN ENUGU STATE

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ABSTRACT

Between June 2008- May 2009 we analyzed the antibiotic susceptibility profile of one hundred and fifty (150) *K. pneumoniae* strains isolated from different clinical samples (urine= 72, high vaginal swab (HVS)=12, sputum=50 and wound swab= 16) from patients visiting University of Nigeria teaching hospital (UNTH) Enugu. All samples were analyzed and organisms isolated using standard Microbiology techniques, antibiotic susceptibility testing was carried out as described in the manual of antibiotic susceptibility testing. Clonal relatedness of resistance strains of *K. pneumoniae* from different samples was determined by randomly amplified polymorphic DNA (RAPD). Antibiotic susceptibility studies revealed that *Klebsiella pneumoniae* strains from wound samples were the most susceptible strains followed by HVS, sputum and urine. The overall susceptibility proflie are as follows; imipenem (100%), amikacin (100%), cefoxitin (99.4%), aztreonam (98%), ceftazidime (98%), cefotaxime (96.7%), amoxicillin/clavulanic acid (96%), ciprofloxacin (96%), tobramycin (93.3%), kanamycin (90%), cefuroxime (86.7%), gentamicin (76%), sulphamethoxazole/trimethoprim (22%), chloramphenicol (15.4%) and ampicillin (5%). RAPD analysis to determine the clonal relatedness of resistance strains grouped them into two clusters (A and B) based on band patterns. All strains resistant to ampicillin and chloramphenicol showed 100% similarity in band patterns (clonal group A) while strains resistant to sulphamethoxazole/trimethoprim showed different band patterns (clonal group B). Our findings revealed high resistant ampicillin, chloramphenicol and sulphamethoxazole/trimethroprim *Klebsiella pneumoniae* strains from different clinical strains belonging to two clusters. These resistant strains may have some public health implications as their spreading is not from single source.

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INTRODUCTION

Klebsiella spps., particularly *K. pneumoniae* is a common hospital-acquired pathogen that causes nosocomial infections such as pneumonia (lung infections), wound infections, meningitis, abscesses, urinary tract infections and diarrhea (Paterson *et al.,* 2007), with neonates and immune-compromised hosts patients as the people at risk. The genus *Klebsiella* comprises of five species, *K. pneumoniae, K oxytoca, K. planticola, K. terrigena* and *K. ornithinolytica* (Bruce 1996), which are usually identified and differentiated according to their biochemical reactions. *K. pneumoniae* is the most common strain found in hospital patients and has been reported to cause out break of sepsis and death of newborns in the intensive care unit of a tertiary hospital like in Brazil (Otman *et al.,* 2002), in the United Kingdom hospitals (Johnson *et al.,* 1992), in France (Arlet *et al.,* 1990).

The discovery of antimicrobial agents had a major impact on the rate of survival from infections; however, the changing patterns of antimicrobial resistance have caused a demand for new antimicrobial agents. Antimicrobial resistance is known to have a very serious clinical and public health implications (Oteo *et al.,* 2002). The wide spread use of broad-spectrum antibiotics had led to the emergency of nosocomial infections caused by drug resistant microbes (Chikere *et al.,* 2008). This is a world wide problem that is exacerbated by the limited number of new antimicrobial drugs (Spellberg *et al.,* 1998; Talbot *et al.,* 2006). Microbial resistance to antibiotics can be by the following ways; (a) drug inactivation by degradation or enzyme modification such as beta lactamases and aminoglycosides transferase (Kiratisin *et al.,* 2008), alteration of drug targets (Kusum *et al.,* 2004), emergency of a bypass pathway not inhibited by the drugs (Xiong *et al.,* 2002) and reduced membrane permeability of the drug (Wang *et al.,* 2008). Resistance due to drug efflux can result in multi-resistance due to the presence of antibiotic resistant genes in the chromosome or plasmid within the intergrons which helps in horizontal transfer of resistance. Bacteria infections caused by *Klebsiella* spps. are often treated with beta-lactam antibiotics or alternatively with aminogycosides or floroquinolones but prevalent of strains resistance to this selected antibiotics had been reported (Brui-Buisson *et al.,* 1987; Sirot *et al.,* 1988; Sekowska *et al.,* 2002). Bearing this in mind we therefore embarked on the present study to determine the antibiotic susceptibility of *Klebsiella pneumoniae* strains isolated from different clinical specimen in a University teaching hospital.

MATERIAL AND METHODS

Study population

Clinical samples were collected from a total of 390 patients (male=176 female=214) attending University of Nigeria teaching hospital (UNTH) Ituku Ozalla in Enugu capital city in South-Eastern Nigeria from June 2008 through May 2009. Clinical samples were obtained by informed consent of patients used for the study with the permission obtained from the ethical committee of the hospital.

Sample collections

Non-repetitive clinical samples which include urine (126), high vaginal swab (68), wound swab (90) and sputum (106) were collected from 390 patients and were analyzed between 20- 45 mins of collection. These clinical samples were analyzed using standard routine Microbiology identification and characterization methods as described in Manual of Clinical Microbiology. After identification and characterization of the 390 samples, *K. pneumoniae* was isolated from 150 samples (urine 72, HVS 12, wound swab 16, sputum 50) based on their apperance in agar plates, biochemical test results and was stored as a glycerol stock culture in a freeze at -20 ^{o}C for further analysis (Farmer 1999).

Antibiotic susceptibility testing

Antibiotic susceptibility of *K. pneumoniae* to commonly used antibiotics in the hospital was determined using the agar-diffusion methods on Mueller-Hinton as described in the Manual of

antibiotic susceptibility testing (Coyle, 2005). Each organism was inoculated into 5 ml of nutrient broth and incubated at 37 ^{o}C for 18-24 hrs, the broth culture was diluted with sterile water to 0.5 MacFarland standards which was uniformly spread on the surface of Mueller-Hinton agar plates using sterile cotton buds. The plates were inoculated with the following antibiotics after 15 mins of inoculating the test organism; ampicillin, ceftazidime, cefotaxime, cefoxitin, cefuroxime, aztreonam, amoxicillin/clavulanic acid, imipenem, chloramphenicol,ciprofloxacin, sulphamethoxazole / trimethoprim, amikacin, kanamycin, tobramycin and gentamicin. Inoculated plates were incubated at 37 ^{o}C for 18-24 hrs after which the inhibition zone diameter was taken. Susceptibility and resistance was determined using the National Committee for Clinical Laboratory Standard breakpoints (CLSI 2007).

DNA isolation

Genomic DNA of all strains resistant to ampicillin,chloramphenicol and sulphamethoxazole /trimethoprim was prepared using the Nucleospin Kit (Macherey & Nagel, Germany) following manufacturer's instructions. An overnight culture in a fresh Luria Bertani broth incubated at 37 ^{o}C for 18-24 hrs was prepared of all *K. pneumoniae* strains. A 1.5 ml of this overnight broth culture was transferred into a reaction tube and centrifuged for 5 mins at 8,000 rpm and supernatant discarded. Pre-lysis was carried out by re-suspending the pellet in 180 µl of T_1 buffer and 25 µl of proteinase K, mixed vigorously and incubated at 56 ^{o}C for 30 mins with shaking. 200 µl of B_3 buffer was added and incubated at 70 ^{o}C for 10 mins. 210 µl of 96-100% ethanol was added into tube containing 200 µl of B_3 buffer and was mixed vigorously until all insoluble particles becomes soluble. This solution was transferred into a Nucleospin column and centrifuged for 1 min at 11,000 rpm, flow through was discarded and the column was placed back into the collection tube. 500 µl of BW buffer was added and centrifuged at 11,000 rpm for 1 min, flow-through was discarded and 600 µl of B_5 buffer was added, centrifuged for 1 min at 11,000 rpm, then flow through was discarded and the column was centrifuge again for 1 min at 11,000. Elution buffer was pre-incubated for 5 mins at 70 ^{o}C and 100 µl was added into each column and centrifuged for 1 min at 11,000 rpm to elute the total DNA. Eluted total DNA was stored at -20 ^{o}C for further analysis.

Randomly amplified polymorphic DNA (RAPD) of resistance *k. pneumoniae* strains

RAPD was performed with all *K. pneumoniae* strains resistant to ampicillin, chloramphenicol and sulphamethoxazole/trimethoprim using a single primer. The PCR mixture contained 2.5 µl each of buffer, 4.0 mM each of dNTP, 2.5 µM each of primer, 5.0 µl each of genomic DNA, 2U each of Taq polymerase, 1.5 µl of $MgCl_2$ and 9.5 µl of water in a total of 25 µl with the following PCR amplification protocol; initial denaturation at 94^{o}C for 5 mins, followed by 34 cycles of denaturation at 94^{o}C for 1 min, 36 ^{o}C for 1 min, 72 ^{o}C for 2 mins and final extension step of 72^{o}C for 8 mins. Amplified PCR products were separated on 1.5 % agarose gels at 75 Volts, stained with ethidium bromide and visualized under UV illumination (Vogel *et al.,* 1999).

RESULTS

Table 1, shows the frequency of isolation of *K. pneumoniae* from various clinical specimens under study. Of the 390 clinical samples analyzed for the presence of *K. pneumoniae,* 150 were positive which include urine= 72, HVS = 12, wound sample = 16 and sputum = 50. The overall prevalence of *K. pneumoniae* in the clincal samples were 38.5% with female 21.5% and male 16.9%. Strains of *K. pneumoniae* isolated from wound swabs were the most susceptible strains followed by HVS and sputum samples while those from urine samples were the least susceptible Table 2. The overall susceptibility of *K. pneumoniae* to different antibiotics are as follows;
Imipenem (100%),
Amikacin (100%),
Cefoxitin (99.4%),
Ceftazidime (98%),
Aztreonam (98%),
Cefotaxime (96.7%),
Ciprofloxacin (96%),
Moxicillin /clavulanic acid (96%),

Tobramycin (95.4%),
Kanamycin (90%),
Cefuroxime (86.7%),
Gentamicin (74%),
Sulphamethoxazole/trimethoprim (48%),
Chloramphenicol (15.4%) and
Ampicillin (5%).

K. pneumoniae strains were highly resistant to ampicillin and chloramphenicol while moderate resistance was observed with sulphamethoxazole /trimethoprim (Fig 1.). RAPD analysis to determine the clonal relatedness of resistance strains of *K. pneumoniae* from various clinical samples grouped our strains into two clonal groups (A and B) based on band pattern. All strains resistant to ampicillin and chloramphenicol showing the same band patterns were grouped as clonal A while those resistant to sulphamethoxazole/trimethoprim with different band patterns are grouped as clonal B.

DISCUSSION

The rate at which antibiotic resistance is been reported in different parts of Nigeria is alarming. Adeyemo *et al.*, 1994, reported the high resistance of *K, pneumoniae*, *E. coli, Peudomonas* spp and *Proteus* spp isolated from children with UTI infections to ampicillin and co-trimoxazole. Omonigbo *et al.,* 2001, reported on resistant urinary isolates of *Escherichia coli* and *K. pneumoniae* to Nalidixic acid, Onifade *et al.,* 2005 reported bacteria susceptible to various classes of antibiotics isolated from pregnant women in ondo State, Aiyegoro *et al.,* 2007, reported the presence of resistant bacteria isolated from children and adolescents in IIe-Ife, Nigeria, Chikere *et al.,* 2008 reported resistant organisms isolated from patients in government hospital in Port-Harcourt, and Okonko *et al.,* 2009 reported bacteria highly resistant to ampicillin, chloramphenicol and tetracycline isolated from

Table 1. Percentage frequency of isolation of *K. pneumoniae* strains from different clinical specimen

Clinical specimens	Number of specimen collected	Number of *K. pneumoniae* strains isolated
Urine	126	72
Sputum	100	50
High vaginal swabs	68	12
Wound swabs	90	16
Total	390	150

Table 2. Percentage susceptibilty of *K. pneumoniae* strains isolated from different clinical specimen

Antibiotics Names												
Amp	Ctx	Caz	Cxm	Ipm	Atm	Amc	Fox	Ak	Cn	K	Tob	Cip
Klebsiella pneumoniae strains isolated from urine specimens												
0	95.8	98.6	84.7	100	98.6	93	100	100	76.4	88.8	76.5	98.6
Klebsiella pneumoniae strains isolated from sputum specimens												
6	94	100	88	100	100	94.1	98	100	74	90	81	90
Klebsiella pneumoniae strains isolated from wound swab specimens												
0	100	100	93.8	100	100	100	100	100	75	87.5	93	93.8
Klebsiella pneumoniae strains isolated from HVS specimens												
8.3	91.6	91.6	100	100	100	83.3	100	100	75	83.3	68	75

Keys: amp-ampicillin, ctx-cefotazime, caz-ceftazidime, cxm-cefuroxime, ipm-imipenem, atm-aztreonam, amc-amoxicillin/clavulanic acid, fox-cefoxitin, ak-amikacin, cn-gentamicin, k-kanamycin, tob-tobramycin, cip-ciprofloxacin, chl-chloramphenicol, sxt-sulphametoxazole/trimetroprim

clinical samples in Abeokuta. Their reports revealed a tremendous increase in antibiotic resistance in hospital form different parts of Nigeria and this can be inferred to the mis-use of antibiotics mainly due to lack of control body to regulate antibiotic use. In developing countries like Nigeria, in-appropriate use of antibiotics is common and this usually leads to resistance development in bacteria previously known to be susceptible. Also in Nigeria, self medication is a common practice and this might probably be one major cause of antibiotic resistance in clinical isolates since patients only think of going to the hospital when they can no longer treat themselves. Taking of expired antibiotics, counterfeit drugs coupled with inadequate hospital control measures that are common practice can as well promote the development of resistance in clinical isolates (Chikere *et al.,* 2008).

A total of 150 clinical isolates of *K. pneumoniae* were isolated from 390 different clinical specimen analyzed which include urine, wound swab, HVS and sputum collected from UNTH Enugu during a twelve months study period. *K. pneumoniae* was predominantly isolated from urine samples 72(57.1%) followed by sputum 50(47.1%) wound swab 16(17.7%) and HVS 12(17.6%). Their susceptibility testing against 15 different antibiotics revealed high susceptiblility to majority of the antibiotics namely; imipenem, amikacin, cefoxitin, ceftazidime, aztreonam, cefotaxime, ciprofloxacin, amoxicillin /clavulanic acid, tobramycin, kanamycin, cefuroxime, gentamicin and resistant to ampicillin, chloramphenicol and sulphamethoxazole /trimethoprim. This result is similar to that reported by Aiyegoro *et al.,* 2007 who reported *Klebsiella* spps. resistance to amoxicillin and co-trimoxazole. Another study in Israel reported a multi-resistance *Klebsiella* spp in a neo-natal intensive care (Leavitt *et al.,* 2007).

Resistance of *K. pneumoniae* to this ampicillin is not surprising because *K. pneumoniae* has intrinsic resistance to ampicillin (Farmer 1999). *K. pneumoniae* is a gram-negative bacterium belonging to the enterobactericeae family and they are known for their high resistance to various antibiotics. This organism harbors series of antibiotic resistance genes which can be transferred horizontally to other bacteria spps (Piddock 1996), it has been implicated in series of nosocomial infections out break in hospitals (Chikere *et al.,* 2008; Lewis *et al.,* 2007). Amikacin and imipenem are the most effective antibiotics being 100% active against *K. pneumoniae* strains, this may be that these antibiotics have not been extensively used to cause resistance developing against them by acquiring resistant genes. Beta- lactam antibiotics are know to be the most widely used antibiotics but our strains were still highly susceptible to the 2^{nd} ,3^{rd} and beta lactam inhibitors used in the study. This will also be attributed to the fact that these organisms are not harbouring resistance genes like the extended spectrum beta lactamase enzymes but resistance to ampicillin, chloramphenicol and sulphamethoxazole /trimethoprim may be attributed to the presence of beta lactamses enzymes that are know to detoxify the penicillins. These antibiotics are cheap and can always be found over the counter in the pharmacy and the lack of control in the use of antibiotics in Nigeria have lead to their mis-use both in the hospital and within the community as a result leading to the present resistance observed.

The frequent un-controlled use of antibiotics in Nigeria has resulted to grave resistance development as have been reported in some previous studies (Onifede *et al.,* 2005; Aiyegoro *et al.,* 2007; Chikere *et al.,* 2008; Okonke *et al.,* 2009). Using RAPD PCR and response of *K. pneumoniae* strains to 15 different antibiotics gave us a method of presumptively identifying clonal groups. We identified two prevalent clones based on this method: one group with resistance to ampicillin and chlorapmphenicol and the other group with resistance to sulphamethoxazole /trimethoprim. This was inferred based on band patterns apperance of these resistance strains on agarose gel. It is also noteworthy that these resistant strains are not from one common source. The clustering of resistant strains into two different groups as observed could be worrisome and may pose some public health problems in future.

Conclusion

In conclusion, our present study revealed *K. pneumoniae* from different clinical specimen that

are susceptible to a wide range of antibiotics but are highly resistant to ampicillin, chloramphenicol and sulphamethoxazole/trimethoprim belonging to two different clones. This could be of serious public health implication because of the possibility of horizontal gene transfer to other bacteria spps. We therefore advocate for proper use of antibiotics in Nigeria both in the hospital and within the community and also requesting that government should provide a body that will be responsible for regulating the use of antibiotics.

REFERENCES

Adeyemo, A.A., Gbadegesin, R.A., Onyemenem I.N. and Ekweozor C.C. 1994. Urinary tract pathogens and anti-microbial sensitivity in children in Ibadan, Nigeria. *Annals of Trop. Paed.*,14: 271-274.

Aiyegoro O.A., Igbinosa O.O., Ogunmwonyi I.N., Odjadjare E.E., Igbinosa O.E. and Okoh A.I. 2007. Incidence of urinary tract infections (UTI) among children and adolescents in IIe-Ife, Nigeria. *Afric. J. Microbio. Resear.*, 1: 13-19.

Arlet, G., Sanson-le pors M.J., Rouveau M., Fournier G., Marie O., Schlemmer B. and Philippon A. 1990. Outbreak of nosocomial infections due to *Klebsiella pneumoniae* producing SHV-4 beta lactamase. *Eur. J. Clin. Microbiol. Infect. Dis.,* 9(11): 797-803.

Bruce, J. 1996. Automated system rapidly identifies and characterizes microorganisms in food. *Food Techol.*, 50: 77-81.

Brun-Buisson, C., Legrand P., Philippon A., Montravers F., Ansquer M. and Duval J. 1987. Transferable enzymatic resistance to third generation cephalosporins during nosocomial out-break of multi-resistant *Klebsiella pneumoniae.* Lancet (ii) 302-306.

Chikere C.B., Chikere B.O. and Omoni V.T. 2008. Antibiogram of clinical isolates from a hospital in Nigeria. *Afric. J. Biotechnol.*, 7(24): 4359-4363.

Coyle, M.B. 2005. Manual of Antimicrobial Susceptibility Testing. Am. Soc. Microbiol. Press, Washinton, DC 25:39.

Clincal and Laboratory Standards Institute, 2007. Performance standards for antimicrobial susceptibility testing, 17th Informational supplement (M100-517). Wayne pa: Clinical and Laboratory Standards Institute.

Farmer, J.J. 1999. Enterobactericeae: Introduction and identification. In: Murray, RP et al., Manual *of Clinical Microbiology*. 7th ed. Washington DC, American Society for Microbiology, p 442-458.

Johnson, A.P., Weinbren M.J., Ayling-Smith B., Du Bois S.K., Amyes S.G.B. and George R.C. 1992. Outbreak of infection in two hospitals caused by strains of *Klebsiella pneumoniae* resistant to cefotaxime and ceftazidime. *J. Hosp. Infect.,* 20: 97-103.

Ko, W.C., Paterson, D.L., Sagnimeni, A.J., Hansen, D.S., Von Gottberg, A., Mohapatra, S., Casellas, J.M., Goossens, H., Mulazimoglu, L., Trenholme, G., Klugman, K.P., McCormack, J.G. and Yu, V.L. 2002. Community-acquired *Klebsiella pneumoniae* bacteremia: global differences in clinical patterns. *Emerg. Infect Dis.,* 8(2): 160-6.

Kiratisin, P., Apisarnthanarak A., Laesripa C. and Saifon, P. 2008. Molecular characterization and epidemiology of extended-spectrum beta lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates causing health care-associated infection in Thailand, where the CTX-M family is endemic. Antimicrob. Agents Chemother. 52:2818-2824.

Kusum, M., Dhiraputra, W.S., Pongpech P. and Naenna P. 2004. Occurrence of extended-spectrum beta-lactamase in clinical isolates of *Klebsiella pneumoniae* in a University Hospital, Thailand. *J Med. Assoc. Thai.,* 87: 1029- 1033.

Leavitt A., Navon-Venezia S., Chmelnitsky I., Schwaber M.J. and Carmeli Y. 2007. Emergency of KPC-2 and KPC-3 in carbapenem-resistant *Klebsiella pneumoniae* strains in an Israel hospital. Antimicrob. Agents. *Chemother*, 51(8): 3026-3029

Lewis, J.S., Herraera M., Wickers B., Patterson J.E. and Jorgensen J.H. 2007. First report of the emergency of CTX-M- type extendeed spectrum beta lactamases (ESBLs) as the predominant ESBL isolated in a US healthcare system. Antimicrob. *Agents Chemother*, 51: 4015-4021.

Otman, J, Perugini, M.E., Cavasin, E. and Vidotto, M.C. 2002. Study of an outbreak of extended-spectrum beta lactamase-producing *Klebsiella* species in a neonatal intensive care unit in Brazil by means of repetitive intergenic consensus sequence-based PCR (ERIC-PCR). *Infect. Contr. Hosp. Epidem.,* 23: 8-9

Oteo, J., Campos J. and Baquero F. 2002. Antibiotic resistance in 1962 invasive isolates of *Escherichia coli* in 27 Spanish hospitals participating in the European Antimicrobial Resistance Surveillance System. *J. Antimicrob. Chemother,* 50: 945-52.

Onifade, A.K., Omoya, F.O. and Adegunloye, D.V. 2005. Incidence and control of urinary tracts infections among pregnant women attending antenal clinics in government hospitals in Ondo State, Nigeria. *J. Food, Agric. Environ.,* 3(1): 37-38.

Omonigbo, S.E., Obasi E.E. and Akukalia R.N. 2001. *In-vitro* resistance of urinary isolates of *Escherichia coli* and *Klebsiella* species to nalidixic acid. *Nig. J. Microbiol*., 15(1): 25-29.

Okonko, I.O., Soleye F.A., Amusan, T.A., Ogun A.A., Ogunnusi T.A. and Ejembi J. 2009. Incidence of multi-drug resistance (MDR) organisms in Abeokuta, Southwestern Nigeria. *Glo. J. Pharm.,* 3(2): 69-80.

Piddock, L.J.V. 2006. Clinically relevant chromosomally encoded multi-drug resistance efflux pumps in bacteria. *Clin. Microbiol. Rev.,* 19(2): 382-402.

Spellberg, B., Powers, J.H., Brass, E.P., Miller, L.G., Edwards, J.E. Jr, 2004. Trends in antimicrobial drug development: implications for the future. *Clin. Infect. Dis.,* 38: 1279-86.

Sirot, J., Chanal, C., Petit, A., Sirot, D., Labia, R. and Gerbaud, G. 1988. *Klebsiella pneumoniae* and other enterobactericeae producing novel plasmid-mediated beta lactamases markedly active against third-generation cephalosporins: epidemiologic studies. *Rev. Infect. Dis.,* 10: 867-878.

Sekowska, A., Janicka, G., Klyszejko, C., Wojda, M., Wroblewski, M. and Szymankiewicz, M. 2002. Resistance of *Klebsiella pneumoniae* strains producing and not producing ESBL (extended spectrum beta lactamase) type enzymes to selected non-beta lactam antibiotics. *Med. Sci. Monit*., 8(3): BR100-4.

Talbot, G.H., Bradley, J., Sewards, J.E. jr, Gilbert D., Scheld, M., Bartlett, J.G. 2006. Antimicrobial Availability Task Force of the Infectious Disease Society of America.. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Disease Society of America. *Clin. Infect. Dis*., 42: 657-68.

Vogel, L, Jones, G, Triep, S, Koek, A. and Dijkshoorn, L. 1999. RAPD typing of *Klebsiella pneumoniae, Klebsiella oxytoca, Serratia marcescens* and *Psuedomonas aeruginosa* isolates using standard reagents. *Clin. Microbiol. Infect.,* 5(5): 270-27.

Wang, A.Y.Y., Lu, Q., Wang, Y., Chen, Y., Deng, L., Ding, H., Deng, Q., Wang, L. and Shen, X. 2008. Occurrence of qnr-positive clinical isolates in *Klebsiella pneumoniae* producing ESBL or AMPC- type beta-lactamase from five pediatric hospitals in China. *FEMS Microbio. Lett.,* 283: 112-116.

Xiong, Z., Zyang, Y. and Wang, F. 2002. Extended-spectrum beta-lactamase in *Klebsiella pneumoniae* and *Escherichia coli* isolates. Zhonghu*a Yi Xue Za. Zhi.,* 82: 1376-1479.

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