JSR Publications

Available Online

J. Sci. Res. **4** (2), 499-505 (2012)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

Short Communication

Effect of Sodium Dodecyl Sulfate and Acridine Orange on Isolation of Plasmid and Antimicrobial Resistance Pattern of Clinical Isolates of *Klebsiella sp.*

M. Shahriar*, S. Mawla, S. M. A. Islam, and M. A. Bhuiyan

Department of Pharmacy, University of Asia Pacific, Dhanmondi, Dhaka, Bangladesh

Received 23 August 2011, accepted in final revised form 23 April 2012

Abstract

The susceptibility pattern of 25 *Klebsiella* sp. samples, collected for a six months time period from diagnostic centers, was investigated against 16 different antimicrobials. All the isolates of this study exhibit resistance against cephalexin, cephradine, ciporfloxacin, cloxacillin, erythromycin, oxacillin, rifampicin and tetracycline. Most of the isolates were resistant to amoxicillin (92%), vancomycin (96%), neomycin (84%) and chloramphenicol (76%). A few of the isolates showed resistance to tetracycline (36%), ceftriaxone (40%) and gentamycin (56%). Seven multidrug resistant (MDR) out of the 25 isolates were selected and grown in varying concentrations of sodium dodecyl sulfate (SDS) in Luria broth. Their plasmid profile was also analyzed. Distinct plasmid bands were observed when the MDR strains of *Klebsiella sp.* were grown in 5% SDS and acridine orange (10 µg/ml) in Luria broth. One of the isolates (isolate no. 3) treated with 5% SDS and/or 5% SDS along with acridine orange developed sensitivity against ceftriaxone, ciprofloxacin, co-trimoxazole, gentamycin and neomycin.

Keywords: Plasmid; Antimicrobial susceptibility; Multidrug resistant; Sodium dodecyl sulfate (SDS).

doi: http://dx.doi.org/10.3329/jsr.v4i2.8445 J. Sci. Res. **4** (2), 499-505 (2012)

1. Introduction

Klebsiella sp. is well known to most clinicians as a cause of community-acquired bacterial pneumonia, occurring particularly in chronic alcoholics and showing characteristic radiographic abnormalities due to a severe pyogenic infection. In recent years, *Klebsiella* have become important pathogens in nosocomial infections [1]. The polysaccharide capsule of this pathogen, which is the main determinant of their pathogenicity is composed of complex acidic polysaccharides. This massive layer protects the bacterium from phagocytosis by polymorphonuclear granulocytes [2].

*Corresponding address: shahriar_12@yahoo.com

Inappropriate antimicrobial treatment as well as its overuse has become a contributing factor for the emergence of resistant bacteria. It has become a common practice now-a-days to prescribe the antibiotics for the indications in which their use is not warranted, even incorrect or sub-optimal antibiotics are frequently prescribed in the treatment of infections that usually can be resolved without treatment [3]. Accordingly resistance to a single drug can spread rapidly through resistant bacterial population. The increasing antimicrobial resistance currently has been reported for *Klebsiella sp.* as this microorganism becomes highly susceptible to most of the antimicrobials [4].

The extra-chromosomal bacterial plasmid DNA often carry the genes that can provide resistance to naturally occurring antibiotics in a competitive environmental niche, or alternatively their transcripted protein products may act as toxins under similar circumstances. Cell lysis is vital for the extraction of DNA, RNA and proteins from cells. Therefore, it is needed in most cell biology techniques and even molecular biology techniques.

The present study is aimed 1) to determine the resistance pattern of *Klebsiella sp.* against antimicrobials, 2) to correlate this resistance pattern with their plasmid profile, 3) to elaborate the effect of sodium dodecyl sulphate (SDS) and acridine orange on plasmid isolation along with a better understanding on antimicrobial resistance pattern of multi-drug resistant (MDR) *Klebsiella sp.* in clinical setup /or clinical specimen and also 4) to carry out an investigation for the antibiotics that the microorganism is sensitive for.

2. Materials and Methods

Source of sample collection: Twenty five isolates of *Klebsiella sp.* were collected from pathological specimens of different diagnostic laboratories over a six months time period. The samples were collected in McConkey agar slunt in test tubes and brought to the research laboratory and further incubated at°C37for 18 hrs. The presence of gram negative bacteria was depicted with red colonies in the media.

Antimicrobial susceptibility test: The disc diffusion method as described by Kirby Bauer was adopted to assay the antimicrobial susceptibility pattern of *Klebsiella sp.* to 17 antibiotics [5,6] namely amoxicillin, Am (30mcg/disc), ceftriaxone, Ci (30mcg/disc), cephalexin, Cp (30 mcg/disc), cephradine, CV (25 mcg/disc), chloramphenicol, C (30 mcg/disc), ciprofloxacin Cf (5 mcg/disc), cloxacillin CX (1mcg/disc), co-trimoxazole, Co (25 mcg/disc), erythromycin, E (15 mcg/disc), gentamycin G (10 mcg/disc), neomycin N (30 mcg/disc), oxacillin OX (1 mcg/disc), penicillin G P (10 mcg/disc), rifampicin R (5 mcg/disc), tetracycline T (30 mcg/disc) and vancomycin VA (30 mcg/disc). All tests were performed in Mueller-Hinton Agar plates.

From the first antibiogram, 7 MDR samples from the *Klebsiella sp.* isolates were selected and each of them was treated with 0.1%, 1% and 5% SDS, respectively. Antibiotic susceptibility test for these samples was carried out in absence of SDS and each of those isolates were treated with 0.1%, 1% and 5% SDS by disc diffusion Kirby Bauer method [5,6] using the same 17 antimicrobials. The plasmid profile analysis of the above samples was done using gel electrophoresis.

Plasmid isolation: To isolate plasmid DNA from bacteria, the miniprep alkaline lysis procedure is used [7]. This method was carried for liquid cultures of the MDR and sensitive *Klebsiella sp.* isolates containing 0.1%, 1% and 5% SDS respectively and the result of their plasmid isolation was observed under ultra violet (UV) light.

Separation of plasmid DNA by agaroseggel electrophoresis: Agarose gel electrophoresis [7] method was used to separate Plasmid DNA obtained from the isolates. The plasmid DNA was subjected to electrophoresis in 1.5% Agarose in TBE buffer for about 90 minutes to allow the loading dye in each sample to migrate near the bottom of the gel. The gel was viewed on an UV light box (short wave, ultraviolet products, Inc., San Gabriel, California, USA, 254nm).The photographs were taken under UV illumination using Sony cyber shot 5.1 megapixel, USA.

3. Result and Discussion

The *Klebsiella sp.* isolated from different pathological specimens demonstrated varying degree of sensitivity pattern to different antimicrobials. Most of the samples exhibited resistance to some commonly used antimicrobials such as amoxicillin, cephalexin, cephradine, chloramphenicol, ciprofloxacin, cloxacillin, co-trimoxazole, erythromycin, neomycin, oxacillin, penicillin G, rifampicin and vancomycin (shown in Fig. 1). Isolates

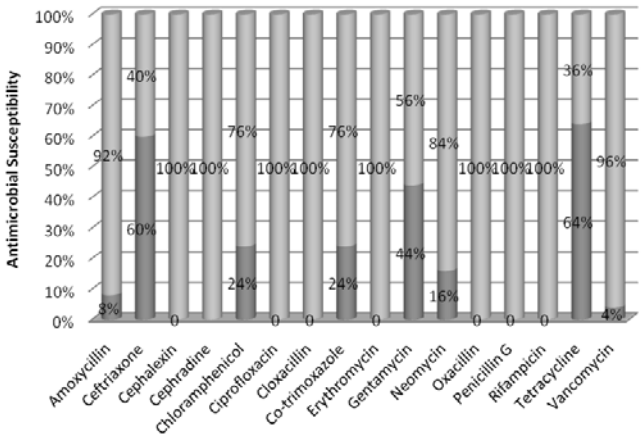

Fig. 1. Antimicrobial susceptibility pattern of isolates of *Klebsiella sp.* Light and darker shading indicate 'resistant' and 'sensitive', respectively.

obtained from urine sample were found to be resistant to antimicrobials. All isolates showed resistance to cephalexin, cephradine, ciporfloxacin, cloxacillin, erythromycin, oxacillin, rifampicin and tetracycline respectively, whereas few showed resistance to tetracycline (36%) and ceftriaxone (40%) and gentamycin (56%). From the study of antimicrobial resistance pattern, 7 MDR isolates were selected (C-437 urine Kleb, Kleb C-

014, C-282 urine Kleb, 512 Kleb, C-579 urine Kleb, 415 urine Kleb and M-204 urine Kleb).

Sample 415 urine Kleb, which is a multidrug resistant *Klebsiella sp.* isolate, developed ceftriaxone, ciprofloxacin, co-trimoxazole, gentamycin and neomycin sensitivity on treatment with 5% SDS and 5% SDS along with acridine orange (10µg/ml).

When cultured with 5% SDS and acridine orange in Luria broth isolate C-437 Urine Kleb also developed resistance to ceftriaxone.

The culture sensitivity pattern (Table 1) of treated (with 5% SDS and acridine orange) and untreated *Klebsiella sp.*to different antimicrobials illustrated that all the 5% SDS and acridine orange treated and untreated isolates collected from clinical scenario were resistant to amoxicillin, cephalexin, cephradine, and cloxacillin.

Table 1. Culture sensitivity pattern of *Klebsiella sp.* to different antimicrobials (grown in absence and presence of 5% SDS and acridine orange in Luria broth).

Sample#	Name of specimen	amoxycillin (Am30)	ceftriaxone (Ci30)	cephalexin (Cp30)	cephradine (CV25)	chloramphenicol (C30)	ciprofloxacin (Cf5)	cloxacillin (CX1)	co-trimoxazole (C025)	erythromycin (E15)	gentamycin(G10)	neomycin(N30)	penicillin G (P10)	rifampicin (R5)	tetracycline (T30)
Antimicrobial sensitivity pattern of *Klebsiella sp.* (not grown in 5% SDS in Luria broth)															
1	Kleb C-014	R	R	R	R	S	R	R	R	R	R	R	R	R	R
2	C-282 urine Kleb	R	R	R	R	S	R	R	R	R	R	R	R	R	R
3	415 urine Kleb	R	R*	R	R	S	R*	R	R*	R	R*	R*	S	S	R
4	C-579 KLEB	R	R	R	R	S	R	R	S	S	R	R	R	R	R
5	C-437 urine Kleb	R	S	R	R	S	R	R	S	S	R	R	S	R	S
6	512 Kleb	R	R	R	R	S	R	R	R	R	R	R	R	R	R
7	Kleb C-36	R	R	R	R	S	R	R	R	R	R	R	R	R	R
Antimicrobial sensitivity pattern of *Klebsiella sp.* (grown in 5% SDS in Luria broth)															
1	Kleb C-014	R	R	R	R	S	R	R	R	R	R	R	R	R	R
2	C-282 urine Kleb	R	R	R	R	S	R	R	R	R	R	R	R	R	R
3	415 urine Kleb	R	S*	R	R	S	S*	R	S*	R	S*	S*	S	S	R
4	C-579 Kleb	R	R	R	R	S	R	R	S	S	R	R	R	R	R
5	C-437 urine Kleb	R	S	R	R	S	R	R	S	S	R	R	S	R	S
6	512 Kleb	R	R	R	R	R	R	R	R	R	R	R	R	R	R
7	Kleb C-36	R	R	R	R	S	R	R	R	R	R	R	R	R	R
Antimicrobial sensitivity pattern of *Klebsiella sp.* (grown in 5% SDS and acridine orange in Luria broth)															
1	Kleb C-014	R	R	R	R	S	R	R	R	R	R	R	R	R	R
2	C-282 urine Kleb	R	R	R	R	S	R	R	R	R	R	R	R	R	R
3	415 urine Kleb	R	S*	R	R	S	S*	R	S*	S	S*	S*	S	S	R
4	C-579 Kleb	R	R	R	R	S	R	R	S	S	R	R	R	R	R
5	C-437 urine Kleb	R	S	R	R	S	R	R	S	S	S	S	S	R	S
6	512 Kleb	R	R	R	R	R	R	R	R	R	R	R	R	R	R
7	Kleb C-36	R	R	R	R	S	R	R	R	R	R	R	R	R	R

R = Showing resistance, **S** = Sensitive, ***** = change in sensitivity to antimicrobials after culturing in presence of 5% SDS and acridine orange (10µg/ml) in Luria broth.

In the present study SDS was used because it is a curing agent and hence it leads to the formation of pores in the bacterial cell wall through which the plasmid come out of the cell which further was verified by gel electrophoresis [8]. Plasmids were identified from the MDR isolates such as Kleb C-014 (1s), C-282 urine Kleb (2s), 415 urine Kleb (3s), C-579 Kleb (4s), 512 Kleb (6s) and Kleb C-36 (7s) cultured in Luria broth with 5% SDS. Similarly plasmids were also isolated from Kleb C-014 (1As), C-282 urine Kleb (2As), C-579 Kleb (4As) and Kleb C-36 (7As) isolates which were cultured in Luria broth culture media in presence of 5% SDS and acridine orange. Although similar sized plasmids were isolated from all of these MDR isolates, it does not confirm the presence of responsible gene in the particular plasmid DNA. As *Klebsiella sp*. isolate, 415 urine Kleb developed sensitivity against ceftriaxone, ciprofloxacin, co-trimoxazole, gentamycin and neomycin, when treated with SDS and acridine orange in supplement with the growth media, possibly this isolate has lost its plasmid in the treatment environment (Fig. 2). This finding positively allows to hypothesize that ceftriaxone, ciprofloxacin, co-trimoxazole, gentamycin and neomycin resistant genes are present in plasmid DNA of the bacteria. Accordingly it can also be assumed that plasmid DNA may be the limiting factor for developing multi-drug resistance for the isolate 415 URINE KLEB. However, in other isolates presence or absence of plasmid showed no relation with the development of ceftriaxone, ciprofloxacin, co-trimoxazole, gentamycin and neomycin resistance. Therefore, further detailed study may be necessary to confirm this issue.

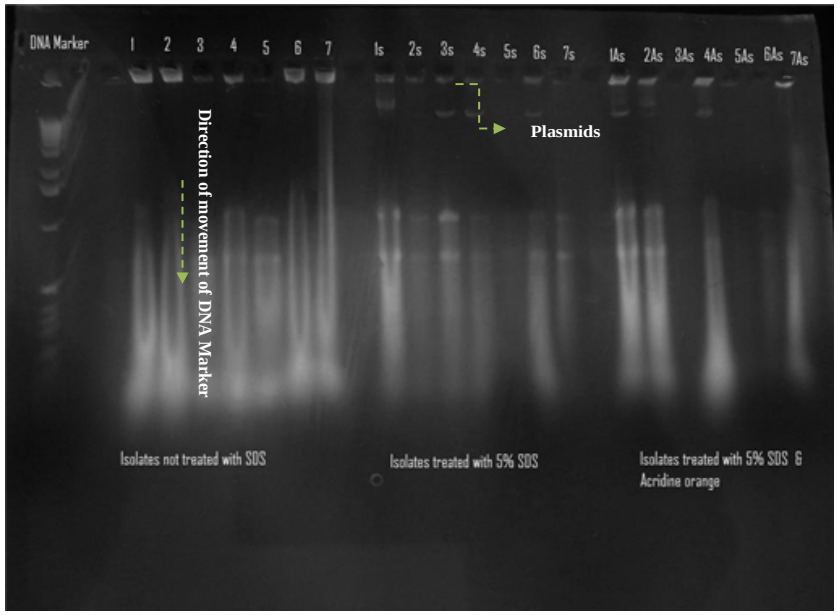

Fig. 2. Electrophoretic patterns showing plasmid DNA in multi-drug resistant isolates of *Klebsiella sp.* grown in 5% SDS and acridine orange.

Although there was no change in the antimicrobial susceptibility pattern of Kleb C-014, C-579 Kleb, and 512 Kleb when grown in 5% SDS, presence of plasmids were observed in respective *Klebsiella sp.* samples. There was no significant change in the result of plasmid profile analysis of *Klebsiella sp.* when grown in acridine orange (10µg/ml) along with 5%SDS.

Drug resistance in microorganisms is a predictable and perhaps inescapable response to the use of antimicrobial agent. It can arise from the selection of resistant strains among naturally susceptible species or from the ingress of new strains of naturally resistant species [9]. The extent of use of particular agents in a given environment dictates the rate at which resistance arises among microbial populations. In the last several years, the question has come up whether it is necessary to determine if each isolated *Klebsiella sp.* is plasmid-mediated extended-spectrum β-lactamase (ESBL) producer [10]. The answer depends on the epidemiologic situation of a country or a hospital, but it should definitely be positive if high percentage of ceftazidime-resistant strains is to be expected. One of the two most commonly used diagnostic tests for the detection of such isolates is the double-disc synergy test where a disc of clavulanic acid and a disc of an extended-spectrum cephalosporin such as ceftazidime are placed close together on an agar surface inoculated with the test organism [10].

In the present study, *Klebsiella sp.* isolates were collected from different pathological specimens and 17 different antimicrobial agents were used to test the susceptibility accordingly. Most of the *Klebsiella* isolates of this study showed resistance to the first line antibiotics that are commonly prescribed by the physicians. Significant number of the samples showed resistance to some commonly used antimicrobials even those, obtained from urine were found to be resistant to antimicrobials. All isolates showed resistance to cephalexin, cephradine, ciporfloxacin, cloxacillin, erythromycin, oxacillin, rifampicin and tetracycline (Fig. 1). Lowest number of isolates showed resistance to tetracycline (36%) and ceftriaxone (40%) and gentamycin (56%).

A change that was observed in our present study is that plasmid isolation was more effective when the isolates were grown in presence of SDS. The results of plasmid analysis varied with different concentrations of SDS in Luria broth (Table 1). SDS and acridine orange (10 µg/ml) in the growth media of *Klebsiella sp.* isolates showed no specific impact on plasmid isolation. An attempt was taken to carry out plasmid curing but it was rendered unsuccessful in this study.

Resistance factors, particularly those carried on mobile elements, can spread rapidly within human and animal populations. MDR pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts. Antibiotic resistance is frequently determined by genetic information of plasmid origin [11]. Previous studies are also there to correlate between the plasmid and resistance of clinical isolates [12]. The correlation between antibiotic resistance and plasmid profile may indicate that the genetic information is plasmid borne. Initially, there was a tendency to assume that antibiotic resistance genes appeared only after antibiotics began to be used

widely in medicine. However, the genetic diversity within some classes of resistance makes it clear that the genes have been evolving for a much longer time [11].

Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections [13]. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs.

4. Conclusion

The antimicrobial resistance of *Klebsiella sp.* to multiple numbers of antimicrobials has been found to increase in comparison with previous studies. To avoid the anomalies in future, antimicrobial drugs should be carefully prescribed only after confirmed pathological investigation and hence rational use of antimicrobials is to be ensured. As this study demonstrated a good relation between bacterial plasmid profile and antibiotic resistance in *Klebsiella* isolates collected from different clinical scenario, further extended studies are thus recommended to confirm whether this resistance is due only to plasmid or to plasmid-mediated extended-spectrum β-lactamases (ESBLs).

References

1. P. Nordmann, G. Cuzon, and T. Naas, Lancet Infect Dis. **9**, 228 (2009). http://dx.doi.org/10.1016/S1473-3099(09)70054-4
2. Y. R. Chan, J. S. Liu, D. A. Pociask, M. Zheng, T. A. Mietzner, and T. Berger, J. Immunol. **182**, 4947 (2009). http://dx.doi.org/10.4049/jimmunol.0803282
3. T. G. Slama, A. Amin, S. A. Brunton et al, Am. J. Med. **118** (suppl. 7A), 1S (2005). http://dx.doi.org/10.1016/j.amjmed.2005.05.007
4. B. Murray, J. Infect Dis. **63**, 1185 (1991). http://dx.doi.org/10.1093/infdis/163.6.1185
5. A. Bauer, W. M. J. Kirby, C. Sherris, and M. Truck, Am. J. Clin. Patho. **44**, 493 (1966).
6. National committee for clinical laboratory standard: Methods for dilution antimicrobial tests for bacteria that grow aerobically, 3rd Edition, approved standard (NCCLS, Pennsylvania, Document M7-A3, 1999).
7. J. Sambrook and D. W. Russell, Molecular cloning: a laboratory manual (Cold Spring Harbor Laboratory, New York, 2001).
8. M. H. Akhter, A. Hussain, and S. N. Khan, Bangladesh J. Microbiol. **22** (1), 55 (2005).
9. C. K. Liam, K. H. Lim, and C. M. Wong, Respirology **6** (3), 259 (2001). http://dx.doi.org/10.1046/j.1440-1843.2001.00336.x
10. R. Podschun and U. Ullmann, Clin. Microbiol. Rev. **11 (**4), 589 (1998).
11. T. T. Myaing, A. A. Saleha, A. K. Arifah, and A. R. Raha, Applications of gene-based technologies for improving animal production and health in developing countries (Printed in the Netherlands, 2005) pp. 521-527. http://dx.doi.org/10.1007/1-4020-3312-5_37
12. M. Shahriar and N. Z. Khair, J. Bacteriol. Res. **3**, 1 (2011).
13. J. Sedor and S. G. Mulholland, Urol. Clin. North Am. **26** (4), 821 (1999). http://dx.doi.org/10.1016/S0094-0143(05)70222-6