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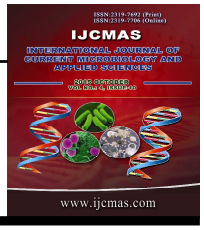

Original Research Article

Demographical Study of Extensive Drug-Resistant Gram-Negative Bacteria with Precise Attention on XDR Uropathogen *E.coli*

Sharmila Devi Natarajan[1], ViswadevanViswajith[2], MahendranKandhasamy[2], Mythili Ravichandran[1], Prathaban Munisamy[1] and Varadharaju Chandrasekar[1]*

[1]Department of Microbiology, K.S. Rangasamy College of Arts and Science, Tiruchengode – 637 215, Tamil Nadu, India
[2]Department of Microbiology, Bioline Laboratory, Cowley Brown Road, R.S. Puram, Coimbatore – 641 002, Tamil Nadu, India
**Corresponding author*

Keywords

Antibiotic resistant, Extensive Drug-Resistant (XDR) Gram-negative bacteria, Epidemiology, Resistance pattern

A B S T R A C T

Objectives of the study is to represent the epidemiological status of progressively increasing XDR Gram-negative bacteria with a distinct attention on XDR Uropathogen *Escherichia coli* in South India. To determine the status of increasing resistant attributes, a broad-level screening was conducted for Gram-negative bacteria by acquiring common infective sample sources including urine, blood, pus, and swab. Standard microbiological tests were conducted for the identification and an antibiotic sensitivity assay was carried out using Osiris automated system. In addition, Vitek 2 Compact was used for further confirmation. Percentage analysis was performed for the statistical outputs using SPSS v 20.0. The overall incidence of XDR Gram-negative bacteria during the studied 1-year period was 0.2%. The highly predominant uropathogen *Escherichia coli* are ranked first with the highest resistance of 54.7%. The geographical location highlighted with more number of resistant *E. coli* is Madurai region of Tamil Nadu. There is a need for nonstop investigation in clinical laboratories, and strict measures should be immediately taken to update the epidemic status of public health regarding this. And a prerequisite of future research on gene-level resistance bacteria dissemination is to increase the awareness in urban and rural region wise.This study will bring a sound record of enduring status of Gram-negative bacteria resistance dissemination.

Introduction

Gram-negative bacterial infections presenting extreme resistance toward the entire major antibiotics consequenceare increasing worldwide (Stuart and Bonnie Marshall, 2004; Marin H. Kollef et al., 2011).Even though many studies have been reported in Gram-negative bacteria, studies with vast samples are limited. The cause of this problem is not only limited to lactose-fermenting Gram-negative bacteria, an increasing number of non-lactose-fermenting Gram-negative bacteria with hospital-acquired infection are being reported(PinyoRattanaumpawan et al

2013;Robert and Dora Szabo, 2006; Marin H. Kollef et al., 2011). Gram-negative bacteria have many inbuilt resistance-generating mechanism to fight against powerful antibiotics (Engel,2009). Even though many Gram-negative bacteria show with multi-drug resistance (MDR), the most common concern is with two species of *Enterobacteriaceae* family: *E.coli* and *Klebsiella*. And the second most resistant species are the pathogenic nosocomial isolates such as *Pseudomonas* sp. and *Acinetobacter*sp (Stuartand Bonnie Marshall, 2004;PinyoRattanaumpawan et al 2013). Nowadays terms such as MDR, extensive drug resistance (XDR), and pan drug resistance (PDR) are commonly used to exhibit the depth of resistance (Magiorakos et al., 2012). Still, no new or novel antibiotic has been developed againstthese resistant bacteria and this situationis also bringing our world to the pre-antibiotic era (Souli et al., 2008;Christian et al., 2008; RekhaBisht et al., 2009; Maltezou, 2009)

An early report of theCenter for Disease Control and Prevention (CDC, 2005) states that more than 70% of the bacteria that cause infections acquired in hospitals are resistant to at least one of the drugs most commonly used to treat them and it is increasing gradually worldwide without intervention (Peleg and Hooper, 2010). Previous report on bacterial resistance suggested to take attention on two things such as antibiotic agent and resistant gene for effective surveillance and necessary actions(Xiao-Zhe Huang et al., 2012; Levy,2001).

In this study, we conducted a sound investigation with vast samples on Gram-negative bacterial isolates collected through BiolineLaboratory in India to detect the depth of resistance among public health. The surveillanceincluded study of urine, blood, pus, swab and other samples collected for Gram-negative bacteria during the period of January 2014 to December 2014. The samples collected all over South India through collection centers were studied usingstatistical secondary data collection. Resistance pattern against all the major antibiotic agentswas analyzed by antibiotic susceptibility test using Osiris. In this epidemiological study, we analyzedthe results only for the XDR Gram-negative bacteria to understand the dissemination of extreme resistance over the covered geographical region and also to compare the various parameters that are included such as age, gender, geographical region, isolates and their resistance patterns. This epidemiological study was moralistically performed to measure the level of becoming infected with XDR Gram-negative bacteria and their prevalence and risk factors concerned with them.

Materials and Methods

Clinical settings

This surveillance study was conducted atBioline Laboratory and Research Institute in Coimbatore, India. It involved 16 major centers of South India, which covered both rural and urban geographical regions of the South India through institutional collection centers. This study mainly targetedthe patients affected with bacterial infections, and the samples included in this study were urine, blood, pus,swab,among others.

Bacterial isolation and identification

The surveillance of sample collection for the bacterial isolates was limited to urine, blood, pus, sputum and others (drain). Primarily, all the samples were inoculated into the Bi-plate system using Blood and Macconkey agar for the tentative strain differentiation of

Gram-positive and Gram-negative bacteria. Routine standard microbiological and biochemical tests were performed further for the bacterial identification. The isolates that were not identified satisfactorily to the species level were again analyzed using Vitek2 Compact (version6.01; bioMerieux, France). Focusing on the increasing MDR in Gram-negative bacteria worldwide with inadequate therapeutic stage (Christian et al., 2008; Marin H. Kollef et al., 2011) this study targeted only the Gram-negative bacterial infection cases.

Antibiotic sensitivity test and XDR detection

Drug susceptibility test was performed using Osiris as appropriate to the procedures mentioned by the Clinical and Laboratory Standards Institute (CLSI) (Akter et al., 2011). The panel of antibiotics tested against all the Gram-negative bacterial isolates includes penicillin, aminoglycosides, monobactam, cephalosporins, quinolones, carbapenems, tetracyclines, amphenicols, sulfonamides and nitrofurantoin, and the resistance rates of MDR organisms were analyzed using percentage analysis.

Data interpretation and analysis

The clinical characteristics were recorded for the entire samples and this epidemiological data included sample source, age, gender, geographical region, isolate information, and drug susceptibility outcomes toward individual antibiotic. The incidence and frequency of XDR Gram-negative bacteria were analyzed using an independent T test and univariate ANOVA of SPSS, version 20.0, software. The predominant Gram-negative bacteria (*E.coli*) were analyzed specifically based on their highest resistance frequency character compared to all other isolates in our drug susceptibility pattern analysis. Following that, XDR*E.coli*-affected patient population study results were mainly analyzed for age factor and geographical region.

Results and Discussion

Demographics of study

During this epidemiological study period, the total number of samples screened was 42,652 and the distribution of samples was as followed: urine, 31,681;blood, 4,471; pus,4,732; swab, 234; and others, 1,534 (Table 1). Of these, 95 samples (0.2%) were confirmed as XDR Gram-negative bacteria.

In Table2, data are reported regarding the demographics, characteristics, and outcome of XDR isolates that were observed as XDR Gram-negative bacteria. In this study, 46.8% patients were male and 53.2% were female, and the distribution of categorical male and female age factor was compared using the independent T test. The *P*-value was<0.718 and it was not statistically significant with the expected state of male with highest XDR presence. Among the total (*n*=95) EDR Gram-negative bacteria, the sample proportion included76 urine (80.8%), 2 blood (2.12%), 15 pus (15.9%), 0 swab (0%),and 1 others (drain;1.1%) samples. The distribution of XDR Gram-negative bacteria is clearly highlighted in the map with region-wise incidence percentage (Fig. 1).

Table3 denotes the prevalence XDR Gram-negative bacteria in specimens. To the overall distribution of 95 XDR Gram-negative bacteria, the prevalence of *E.coli* (*n*=52) was found in 46 urine samples and 5 pus samples and that of *Pseudomonas* sp.(*n*=8) was found in 6 urine samples and 2 pus samples. Out of 31 samples, the prevalence of *Klebsiella* sp. was found in 23 urine samples, 1 blood sample, 6 pus

samples, and 1 other (drain) samples. Of 2 *Acinetobacter* sp. was seen in one blood and one pus sample. Finally, *Morganella* sp. was found in one urine sample and *proteus* sp. was found in one pus sample. The proportion of the XDR organism frequency was*E.coli*,55.3%;*Klebsiella* sp., 32.9%; *Pseudomonas* sp., 8.5%; *Acinetobacter* sp., 2.12%; *Morganella* sp., 1.1%, and *Proteus* sp.,1.1%.

Resistance pattern

Details of the antibiotics used against the entire XDR Gram-negative isolates and its resistance pattern toward all the antibiotics are shown in Table 4. Clinically, the most frequent organism XDR *E.coli* showed 100% resistance toward cephalosporins (cefixime, cefoperazone, cefpodoxime, ceftriaxone and cefuroxime), quinolones (nalidixic acid), and carbapenem (meropenem). Then the highest resistance attributing nosocomial pathogen *Acinetobacter sp*. showed 100% resistance toward penicillins (amoxiclav, piperacillin/ tazobactam, carbenicillin), monobactams, cephalosporins, ciprofloxacin, co-trimoxazole, and carbapenems.

Table. 5 shows the percentage of XDR isolates and their frequency for the whole period (from January 2014 to December 2014) on monthly basis. The results were analyzed using univariate ANOVA analysis and *P*-value was found to be<0.00, which shows nonsignificant status of XDR Gram-negative isolates in the consecutive months of the study period.

Study of XDR: The impact *of E.coli*

The overall *E.coli* population in the resulted XDR Gram-negative isolates is 52 (54.7%) and it is significantly higher than that of other clinically important Gram-negative organisms. Compared to the other samples, it has been predominantly isolated from urine (*n*=47, 90.4%). The gender categorical variables were compared using independent T Test to analyze the higher frequency of XDR *E.coli* isolates among males and females. The *P*-value was <0.158for XDR *E.coli* incidence among male and female patients(Table 6).The percentage of highly watchful *E.coli* in the studied geographical regions is independently depicted in Table 7.

Table.1 Prospective Surveillance of Clinical Samples Incidence and the XDR Gram-Negative Isolates of 2014

Samples	No. of clinical samples	Total no. of XDR Gram-negative isolates of the year 2014
Urine	31,681	*n*= 95
Blood	4,471	
Pus	4,732	
Swab	234	
Others	1,534	
Total	42,652	0.2%

Table.2 Selected Patients Characteristics of Clinically Observed Routine Infections with Extreme Drug Resistance (n=95/42,652) and XDR Patients Age Compatibility Analysis Using Independent T Test

Patient characteristics	Number	Proportion of study population (%)	*P-value
Sex			
Male, casepatients	44	46.8	
Female, casepatients	51	53.6	
Age Group (age at admission)			
0-10 years	16	17.02	<0.718
11-20 years	6	6.38	
21-30 years	10	10.63	
31-40 years	14	14.89	
41-50 years	12	12.76	
51-60 years	15	15.95	
61-70 years	7	7.44	
71-80 years	1	1.06	
81-90 years	6	6.38	
91-100 years	-	-	
Age missing	7		
Infection: sample			
Urine sample	77	81.0	
Blood sample	2	2.12	
Pus sample	15	15.9	
Swab sample	-	-	
Others	1	1.1	

Based on relevance and frequency

Table.3 Frequency of XDR Gram-Negative Bacteria Prevalence in Overall Samples

Clinical specimens	*E.coli* (*n*=52)	*Pseudomonas* sp. (*n*=8)	*Klebsiella* sp.(*n*=31)	*Acinetobacter* sp.(*n*=2)	*Morganella* sp.(*n*=1)	*Proteus* sp. (*n*=1)
Urine(*n*=77)	47	6	23	-	1	-
Blood(*n*=2)	-	-	1	1	-	-
Pus(*n*=15)	5	2	6	1	-	1
Swab (*n*=0)	-	-	-	-	-	-
Others(*n*=1)	-	-	1	-	-	-

Table.4 Percentage Analysis of XDR Resistance Using Major Antibiotics

Antimicrobial agents	**% Resistance (number of 100% resistance isolates/number of tested XDR isolates)**							
	A.baumanii	***E.coli***	***Klebsiella pneumoniae***	***Klebsiella* sp.**	***Klebsiella oxytoca***	***Pseudomonas* sp.**	***Proteus mirabilis***	***Morganella* sp.**
Penicillin								
Amoxyclav	100(2/2)	78.84(41/52)	88.8(16/18)	100(1/1)	66.6(8/12)	50(4/8)	100(1/1)	100(1/1)
Ampicillin/Sulbactam	0(0/2)	73.07(38/52)	77.7(14/18)	100(1/1)	75(9/12)	87.5(7/8)	100(1/1)	100(1/1)
Piperacillin /Tazobactam	100(2/2)	44.23(23/52)	44.4(8/18)	100(1/1)	58.3(7/12)	37.5(3/8)	100(1/1)	0(0/1)
Carbenicillin	100(2/2)	-	100(5/5)	100(1/1)	100(2/2)	50(1/2)	100(1/1)	-
Aminoglycosides								
Amikacin	0(0/2)	46.15(24/52)	61.1(11/18)	100(1/1)	33.3(4/12)	62.5(5/8)	0(0/1)	0(0/1)
Gentamicin	50(1/2)	46.15(24/52)	72.2(13/18)	100(1/1)	66.6(8/12)	50(4/8)	0(0/1)	0(0/1)
Netillin	50(1/2)	42(21/50)	61.1(11/18)	100(1/1)	41.6(5/12)	85.7(6/7)	0(0/1)	100(1/1)
Tobramycin	0(0/2)	50(2/4)	80(4/8)	100(1/1)	50(1/2)	50(1/2)	0(0/1)	-
Monobactam								
Aztreonam	100(2/2)	88.23(45/51)	88.8(16/18)	100(1/1)	91.6(11/12)	75(6/8)	100(1/1)	100(1/1)
Cephalosporins								
Cefaclor	100(2/2)	98.07(51/52)	100(18/18)	100(1/1)	100(12/12)	100(8/8)	100(1/1)	100(1/1)
Cefepime	100(2/2)	80.7(42/52)	94.4(17/18)	100(1/1)	83.3(10/12)	100(8/8)	100(1/1)	100(1/1)
Cefixime	100(2/2)	100(52/52)	100(18/18)	100(1/1)	100(12/12)	100(8/8)	100(1/1)	100(1/1)
Cefoperazone	100(2/2)	100(52/52)	100(18/18)	100(1/1)	100(12/12)	100(8/8)	100(1/1)	100(1/1)
Cefotaxime	100(2/2)	98.07(51/52)	100(18/18)	100(1/1)	100(12/12)	100(8/8)	100(1/1)	100(1/1)
Cefpodoxime	100(2/2)	100(52/52)	100(18/18)	100(1/1)	100(12/12)	100(8/8)	100(1/1)	100(1/1)
Ceftazidime	100(2/2)	92.3(48/52)	88.8(16/18)	100(1/1)	83.3(10/12)	87.5(7/8)	100(1/1)	100(1/1)
Ceftriaxone	100(2/2)	100(52/52)	100(18/18)	100(1/1)	100(12/12)	87.5(7/8)	100(1/1)	100(1/1)
Cefuroxime	-	100(47/47)	100(13/13)	-	100(10/10)	100(6/6)	-	100(1/1)
Cefazolin	-	-	-	-	-	-	-	-
Quinolones								
Ciprofloxacin	100(2/2)		100(18/18)	100(1/1)	83.3(10/12)	62.5(5/8)	0(0/1)	100(1/1)

		96.15(50/52)						
Co-Trimaxazole	100(2/2)	92.3(48/52)	66.6(12/18)	0(0/1)	90(9/10)	62.5(5/8)	100(1/1)	0(0/1)
Levofloxacin	0(0/2)	38.46(20/52)	55.5(10/18)	0(0/1)	33.3(4/12)	50(4/8)	0(0/1)	0(0/1)
Nalidixic acid	-	100(47/47)	100(13/13)	-	60(6/10)	66.66(4/6)	-	0(0/1)
Norfloxacin	-	85.10(40/47)	76.9(10/13)	-	80(8/10)	66.66(4/6)	-	100(1/1)
Ofloxacin	-	78.2(37/47)	91.6(11/12)	-	60(6/10)	66.66(4/6)	-	100(1/1)
Carbapenem								
Ertapenem	-	-	-	-	-	100(1/1)	-	-
Imipenem	100(2/2)	0(0/52)	0(0/18)	0(0/1)	0(0/12)	12.5(1/8)	0(0/1)	0(0/1)
Faropenem	100(2/2)	80(4/5)	100(5/5)	100(1/1)	50(1/2)	100(2/2)	100(1/1)	-
Meropenem	-	100(9/9)	10(3/3)	-	-	100(4/4)	-	-
Tetracyclines	100(2/2)	53.8(28/52)	38.8(7/18)	0(0/1)	25(3/12)	37.5(3/8)	100(1/1)	100(1/1)
Doxycycline hydrochloride	100(2/2)	20(1/5)	66.6(4/6)	0(0/1)	50(1/2)	100(2/2)	100(1/1)	-
Furantoin								
Nitrofurantoin	-	13.0(6/46)	30.7(4/13)	-	30(3/10)	50(3/6)	-	0(0/1)
Amphenicols								
Chloramphenicol	50(1/2)	0(0/5)	60(3/5)	0(0/1)	50(1/2)	50(1/2)	0(0/1)	-

Antimicrobial resistance pattern; % Resistant (number of resistant isolates / number of tested isolates

Table.5 Incidence of XDR Gram-Negative Isolates Month-Wise Frequency Test Using UnivariateANOVA

Isolates	**% of isolates incidence during the period of 2015 (January–December)**												
	January	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**	***P Value**
E.coli	11.32	5.66	13.20	7.54	11.32	11.32	5.66	3.77	13.20	5.66	3.77	7.54	
Klebsiella sp.	10	0	3.33	10	10	6.66	13.33	10	6.66	6.66	10	13.33	
Pseudomonas sp.	12.5	12.5	0	0	25	0	0	12.5	12.5	12.5	0	12.5	
Acinetobacter sp.	0	0	0	0	0	0	0	50	50	0	0	0	
Morganella sp.	0	0	0	0	0	100	0	0	0	0	0	0	*P<0.00
Proteus sp.	0	100	0	0	0	0	0	0	0	0	0	0	

Table.6 Age Compatibility of XDR E.coli Between Male and Female Patients Using Independent T Test

Patients, Age	**No. of XDR *E.coli*isolates in male**	**No. of XDR *E.coli*isolates in female**	***P*-value**
0-20	4	6	<0.158
21-40	6	7	
41-60	5	9	
61-80	1	4	
81-100	2	3	

Table.7 Study of XDR *E.coli* Isolates Frequency in the Studied Regions

Geographical regions	**No. of *E.coli***	**% of incidence (region wise)**
Dindigul	1	1.9
Erode	5	9.6
HeadOffice	15	28.8
Karur	1	1.9
Madurai	22	42.3
PNPalayam	2	3.8
Pollachi	5	9.6
Trichy	1	1.9

Clinical outcomes

The highest XDR prevalence was reported from the Madurai geographical regionof Tamil Nadu, and the incidence of *E.coli*was high in the Madurai geographical region. The accumulation of total number of XDR Gram-negative bacteria (*n* = 95, 0.2%) in this survey has been found. This surveillance study has accounted the XDR gram-negative prevalence as high because 1 in every 454 sampleshad positive XDR Gram-negative bacteria. The demographic gender data took account of prevalence among females(51, 53.6%) and males(44, 46.3%), and in the case of predominant *E.coli*, it was 38.3% for males (*n* = 18) and 61.7% for females (*n* = 29).The prevalence of Gram-negative isolates during this one-year period was higher with *E.coli*(*n*=52, 54.7%)followed by *Klebsiella* sp.(*n*=31, 32.6%),*Pseudomonas* sp. (*n*=8, 8.4%),*Acinetobacter* sp. (*n*=2, 2.1%), *Proteus* sp. (*n*=1, 1.1%), and *Morganella* sp. (*n*=1, 1.1%).

To the best of our knowledge, this is the first study investigating the prevalence of XDR Gram-negative bacteria in South India with vast sample size. The presence of XDR and PDR Gram-negative bacteria is becoming a common scenario next to that of the MDR Gram-negative bacteria (Souli et al., 2008). The global concern and aim toward these drug-resistant pathogens is conducting survey research locally and internationally for drug development. Unfortunately, the development of novel antibiotics against theseXDR Gram-negative bacteria does not meet safety standards (Maltezou, 2009). According to the susceptibility variance, only XDR Gram-negative bacteria were focused in this study to compare the resistant attributesfor major antibiotics. The information provided here is based on a sound epidemiological surveillance and it will be an asset to the national surveillance systems.

The first part of the study shows that the prevalence of bacterial infections in female population is 53.2%, it was higher than that in males(46.8%).It is similar to that shown in various reports worldwide. This is mainly due to urinary tract infections among females, since females are more prone than males (Linhares et al., 2013). Screening found highest number of urine samples with E.coli causing Urinary tract infections(Todd et al., 2013).Using independent T test analysis, *P*-value was found to be <0.718,which is statistically not significant.

Most literature revealed that XDR *Enterobacteriaceae* is associated with high mortality among patients, in particular with carbapenem resistance (Christian et al., 2008; Rekha Bisht et al., 2009;Karthikeyan et al., 2010; Schwaber et al., 2008; Cagnacci et al., 2008; Souliet al., 2008). In our study, we found *E.coli*to be the first and most frequently isolated uropathogen (Peleg and Hooper, 2010).*Klebsiella* sp. was found to be the second, *Pseudomonas* sp., the third, and *Acinetobacter* sp., *Proteus* sp., and *Morganella* sp., were the least and fourth isolated pathogens. However, *Acinetobacter* sp. is the least and also the most pathogenic and resistant attributing organism compare to other organisms (Stuart and Bonnie Marshall, 2004).During the last few decades, *K.pneumoniae*has been found to be the most pathogenic and often showing resistant organism among the *Enterobacteriaceae* family (David Paterson, 2006). Next to the *E.coli* and *Klebsiella* sp., the *Pseudomonas* sp.and *Acinetobacter* sp. are the serious nosocomial pathogens with XDR characters. This extreme capability of these two organisms is due to their ability of acquiring intrinsic resistance to many drugs. In addition, the *Acinetobacter* sp. are

repeatedly reported in many literatures to be MDR, XDR, and PDR pathogens, and it is noted with high impact on ICU, long stay among hospitalized patients, and high mortality rate (Stuartand Bonnie Marshall, 2004;Ahmed Hasanin et al., 2014; Tamma et al., 2012;NeleBrusselaers et al., 2011; Marin H. Kollef et al., 2011). The least isolated XDR *Morganella* sp. and *Proteus* sp. are notstudied since they were obtained once during the study period. In this study, in which samples were collected from 15 regions, the maximum prevalence of XDR(39%) wasfound in the Madurai geographical region andthe second maximum was found in the Coimbatore geographical region (35%). This status could be predicted as these two regions are highly developed with more exposures to antibiotics intake among public.

The overall prevalence of XDR in Gram-negative bacteria was found to be higher for nosocomial pathogen *Acinetobacter* sp., and it is frequent in *E.coli*, which is similar to that shown in many reports worldwide(Stuart and Bonnie Marshall, 2004;Todd et al., 2013; Ahmed Hasanin et al., 2014). In this study, we clearly found that *Enterobacteriaceae* are developing and spreading the resistant characters very fast and the prevalence is also higher in the clinical laboratories. Nowadays XDR Gram-negative bacteria are showing default resistance toward cephalosporin groups (Ahmed Hasanin et al., 2014). In this study the *Acinetobacter* sp., *Klebsiella* sp., *Pseudomonas* sp., *Morganella* sp., *Proteus* sp. have 100% resistance toward cephalosporins, and the predominantly frequent XDR Gram-negative bacterium *E.coli* is showing approximately 100% resistance toward cephalosporins. Next to that, notable nosocomial pathogen with high resistant characters is *Acinetobacter* sp., it shows 100% resistance toward carbapenem group antibiotics, which is the last choice (Christian et al., 2008), and also to utmost of the penicillin groups (Engel,2009). In this case, the there is a presence of beta lactamase and carbapenamase enzymes and the upregulated efflux pump mechanisms rule the outer membrane ofthat organism (Nordmann et al., 2012; Engel,2009).

The present study indigenously performed univariate ANOVA for the XDR Gram-negative bacteria incidence on monthly basis throughout the study period. Fortunately, in South India, this trend is not significant as it shows less alarming status with no constant and increasing XDR prevalence in the following months throughout the period.

The study of predominant XDR Gram-negative pathogen *E.coli*with the highest number of urine samples shows the highest rate of incidence as shown in many of our previous reports on the antibiotic resistance in *Enterobacteriaceae*family (Peleg and Hooper, 2010). The independent T test value analysis of XDR*E.coli* patients' age groupresults with non-significant value,since it is commonly higher with female patients. The prevalence of XDR*E.coli* remains highest in the Madurai region with 42.3% and the second highest in the Coimbatore region with 29%.

Our epidemiological study conducted with the aim of a broad-level screening shows the distribution of XDR Gram-negative bacteria in South India. Our investigation also makes public the remarkable status of highly resistant isolates under various categories such as geographical region, age, and gender. This study also repeatedly confirms that *Acinetobacter baumannii* is the most virulent XDR pathogen with high risk of resistance as it shows 100% resistance toward last-choice antibiotic carbapenems (Stuartand Bonnie Marshall, 2004; Engel,2009).The frequency of XDR

uropathogen *E.coli* is also parallel, with many previous studies by showing highest number of recurrent resistant organism compared to all other Gram-negative isolates observed in this study(Todd et al., 2013; Peleg and Hooper, 2010).We strongly suggest that our study will be the promising evidence on the prevalence of XDR Gram-negative bacteria in the South India. Unfortunately, gene-level dissemination was not performed during that period, and it will be the subject of ourfuture epidemiological investigation.

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