http://dx.doi.org/10.5455/sf.98561

Science Forum (Journal of Pure and Applied Sciences)

journal homepage: www.atbuscienceforum.com

Detection of highly resistant *Enterobacteriaceae* (HRE) from hospital isolates within Bauchi metropolis

Nazeef Idris Usman*, Ismail Haruna, Aliyu Adamu

Microbiology Department, Bauchi State University, Gadau, Nigeria

ABSTRACT

The *Enterobacteriaceae* are Gram-negative, facultative anaerobes that fermenting sugars to produce lactic acid. Most prevalent among this family are *Escherichia coli* and *Klebsiella pneumonia,* recently these species developed resistance to many antibiotics with few exceptions among which include Beta lactams antibiotics. This study explores the susceptibility profile of *Enterobacteriaceae* to commonly prescribed antibiotics. A total of 485 *Enterobacteriaceae* isolate mostly *Klebsiella pneumoniae E. coli*, *Proteus species*, *Enterobacter species*, *Salmonella typhi,* and *Shigella* species) from Urine, High Virginal Swab, Endocervical Swab, Sputum, and Blood were collected from the selected hospital within Bauchi metropolis from April to August 2016 to study the Resistant pattern of *Enterobacteriaceae* (HRE) and some extended spectrum β-lactamases (ESBL) producing *K. pneumoniae* among clinical isolates in the hospital setting. Isolates were tested for possible ESBL production and were phenotypically confirmed. The Kirby Bauer disk diffusion method was followed base on Clinical and Laboratory Standard Institute guidelines, Antibiotic susceptibility testing was further conducted. Among the 485 isolate isolates processed, 347 (71.5%) were tested for multidrug resistant while 138 (28.5%) *Klebsiella pneumoniea* were tested for ESBL production. 56 (40.6%) of *K. pneumoniae* are ESBL producers while 82 (59.4%) are non ESBL Producers. The phenotypic assay confirms the presence of extended drug-resistant *K. pneumoniae* with some *Enterobacteriaceae*. There is also a need for further research to identify the effect of infection with ESBL producing bacteria within the study area.

ARTICLE INFO

Article history:
Received 17 July 2021
Received in revised form
01 August 2021
Accepted 01 August 2021
Published 12 October 2021
Available online 12 October 2021

KEYWORDS

Klebsiella pneumonia
Enterobacteriaceae
ESBL
HRE
Bauchi

1. Introduction

Enterobacteriaceae are the most prevalent bacteria that cause nosocomial and community-acquired infections; they are normally found in the intestinal tract, Urinary tract, and some in the mucous membrane as normal flora (Kattel, 2008 and Sahuquillo-Arce, 2014). They are opportunistic pathogens causing infection in immunocompromised individuals. In this part of the country, the recurrent diagnosis of infection caused by enteric bacteria and mismanagement of drugs may be responsible for an increase in the emergence of extensive-resistant strains of bacteria. The changing and spread of multidrug resistant *Enterobacteriaceae* in humans have limited available therapeutic drugs that are used for the treatment of the infection they cause (Boucher, 2009). Betalactameses, once considered as an effective therapy for many infections caused by *Enterobacteriaceae*

* **Corresponding author** Nazeef Idris Usman ✉ iunazeef@gmail.com ✉ Microbiology Department, Bauchi State University, Gadau, Nigeria.

are now technically ineffective for the treatment of resistant strains (Paterson, 2005).

Self-medication and improper diagnosis or administration of drugs have led to an increase in antibacterial resistance. Antibiotic taken without the prescription of a physician has become a culture for curing minor illnesses which are apparent symptoms for a more devastating infection. These have contributed to the reason why almost all antibacterial agents develop resistance to drugs. Antibacterial resistance and production of enzymatic barriers have created Multidrug-resistant Bacteria which subsequently contributed to failures in diagnosis. Most countries have experienced an increase in extended spectrum β-lactamases (ESBLs) producing bacteria.

These call for a need to search for available therapeutic options that may be effective in treating infections caused by *Enterobacteriaceae*. Effective and efficient identification of ESBL strains is crucial for the selection of antimicrobial therapy and infection control measures (Cohen, 2012). This study aimed at detecting highly resistant *Enterobacteriaceae* from some selected hospitals within the Bauchi metropolis with specific emphasis on the most prevalent members *Klebsiella pneumoniae* and *Escherichia coli*.

2. Methodology

2.1. The study area

Three main hospitals (ATBUTH Bauchi, Specialist Hospital, and Reemee Medicare) were selected within the study area for sample collection. Bauchi city lies on the geographical coordinates of 9° 2′ 0″ north of the Equator, 12° 19′ 0″ east of Greenwich Meridian, with a population of 4,676,465 people as at 2006 census (Nigerian Population and Housing Census 2006).

2.2. Isolate collection

A total of 485 Isolate were collected from the selected hospital within the Bauchi metropolis mainly Reemee medicare limited and specialist hospital from November 2016 to March, 2017.

2.3. Isolate processing

All the bacterial isolates were subcultured and incubated at 37°C for 24 hours on MacConkey agar and Blood agar; the discrete colonies were further subjected to phenotypic characterization as a confirmatory test.

2.4. Gram staining

The slides with heat-fixed smear were placed on the staining tray. It was gently flooded with Crystal violet and allowed to stand for a few seconds. The slide was then slightly and gently rinsed with distilled water using a wash bottle. Lugol iodine was used to flood the smear again and allowed to stand for another 1 minute. It was then decolorize using 95% alcohol or acetone for 10 seconds and rinsed immediately with water. Safranin was used to flood the smeared slide then rinsed again after 1 minute. The stained slides were allowed to air-dried and were viewed with an ×100 objective lens using a microscope.

2.5. Biochemical confirmation

2.5.1. Catalase test

Colony from the subcultured plate was smeared on a sterile dry glass slide using a wire loop, a drop of 3% H_2O_2 was placed on the smeared slide. A positive result was identified by the rapid evolution of oxygen within 10 seconds.

2.5.2. Coagulase test

Pure culture from overnight incubation was placed using sterile wire loop in a test tube containing 2 mls of serum, as the bacteria grow they secret Coagulase enzyme which coagulates the serum by activating prothrombin. The tests differentiate *Staphylococcus aureus* from other species of *Staphylococcus.*

2.5.3. Indole test

Using a sterile wire loop an overnight pure culture was inoculated on Tryptone broth and incubated at 37°C for 24 hours. After incubation five drops of Kovac's reagent were placed into the test tube, Indole production is indicated by the production of pink to red color. However, these differentiate *E. coli* from *Klebsiella species.*

2.5.4. Methyl red and Voges Proskauer tests

Two test tubes containing MR-VP Broth were inoculated with pure culture and Incubate at 35°C for up to 24 hours. Five drops of the methyl red indicator solution (C.I. Acid Red 2 or methyl red reagent) was added to the first tube for the Methyl Red test while Barritt's A and Barritt's B reagents. Five percent of alpha-naphthol, followed by 0.2 ml of 40% KOH was added for the second tube for Voges-Proskauer test. A positive reaction is indicated if the color of the medium changes to red within a few minutes for

the methyl red test and red to brown for the Voges-Proskauer test. This test identifies *E. coli* from some *Enterobacteriaceae*.

2.5.5. Citrate test

Tubes containing Simmons citrate agar were inoculated with overnight pure culture using a sterile wire loop lightly on the slant by touching the tip of a needle to a colony that is 24 hours old. It was Incubate at 37°C for 24 hours. The development of a blue color indicates a positive result.

2.5.6. Urease test

Tubes containing Christensen's urea agar were inoculated with overnight pure culture using sterile wire loop and incubate at 37°C for 24 hours, production of urease enzyme is indicated by a color change from an orange color to magenta. The test was used to identify *Proteus species* and *Klebsiella species.* Following the method of (Amraie et al., 2014), a comparative biochemical test for identification of *Klebsiella pneumoniae* was used in this study.

2.6. Antibiotic susceptibility testing (AST)

AST was conducted base on the Kirby and Bauer disk diffusion method under Clinical and Laboratory Standard Institute (CLSI) guidelines. The colonies of the identified *Enterobacteriaceae* were mixed with Brain Heart Infusion broth and inoculated on Mueller-Hinton agar (MHA) plates using sterile swabs; antibiotic discs were placed on MHA and pressed gently. The prepared Petri dishes were incubated at 37°C for 24 hours. Antibiotic discs tested were; ciprofloxacin (30 µg), pefloxacin (30 µg), ofloxacin (5 µg), Septrin (30 µg), Amoxicillin (30 µg), gentamycin (30 µg), Augmentin (5 µg), chloramphenicol (30 µg), Erythromycin (10 µg), streptomycin (30 µg), Ampicillin (30 µg). Values obtained were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2015). The diameter of the zone of inhibition for each antibiotic was measured and interpreted as resistant, intermediate susceptible, or susceptible according to National Committee for Clinical Laboratory (NCCLS) criteria (SNCCL, 2005).

2.7. Phenotypic detection of extended spectrum Betalactameses

Highly resistant *K. pneumoniae* were separated for detection of ESBL Using the Disc screening method and confirmed by combination disc test (CDT) and Double Disc Synergy Test (DDST).

2.7.1. ESBL assay

Klebsiella pneumoniae isolate was inoculated on Muller Hilton agar and ceftriaxone (30 µ) ceftazidime and (30 µ), cefotaxime (30 µ) were placed using aseptic measures; the plate was then incubated at 37°C for 24 hours. Isolates showing inhibition zone size of ≤ 22 mm with ceftazidime (30 µg), ≤ 25 mm with Ceftriaxone (30 µg), and ≤ 27 mm with cefotaxime (30 µg) were interpreted as screening test positive for ESBL production base on NCCLS criteria (SNCCL, 2005).

2.7.2. Combination disc test (CDT)

Petri-dish containing cephalosporin alone (cefotaxime 30 µg) and combination clavulanic acid and Ceftriaxone (30 + 10 µg) were placed on a prepared MHA plate containing the test bacteria and incubated at 37°C for 24 hours. The zone of inhibition combine with Ceftriaxone-clavulanic acid is compared with the zone of cefotaxime alone. The test was considered positive when the inhibition zone diameter is ≥ 5 mm larger with clavulanic acid than without.

2.7.3. Double-disc synergy test (DDST)

Ceftriaxone was placed next to Amoxicillin + Clavulanic acid on a prepared Nutrient Agar plate and incubated at 37°C for 24 hours. The result was considered positive when Ceftriaxone discs zone inhibition augmented in the direction of the disc containing clavulanic acid.

3. Results

Out of the total of 485 isolates were used in this study, *K. pneumoniae* 138 (28.5%), *E. coli* 107 (22%), *Salmonella typhi* 87 (18%), *Shigella* species 77 (15.8%), *Proteus species* 43 (8.9%) and *Enterobacter species* 33 (6.8%). They are identified from Urine, Stool, and Blood samples. The isolate was distributed within 5–82 years, which were more prevalent

Table 1. Distribution of isolate by age.

S/no	Age	Isolate (*n* = 485)	Percentage
1.	5–15	33	6.8
2.	16–25	43	8.9
3.	26–35	107	22.1
4.	36–45	138	28.4
5.	46–55	87	17.9
6.	>56	77	15.9
Total		485	100

within the age of the working class (26–45 years) with a total of 245 (50.5%) and less in the infant ages (5–15) 6.8% as reported in Table 1. From Table 2, among the 485 isolates, 245 (50.5%) were identified from the female patient while 240 (49.5%) were isolated from male patients; the occurrence rate of *K. pneumoniae* 138 (28.5%) was found to be higher among the *Enterobacteriaceae* isolate followed by *E. coli* with 107 (22%). The frequency of highly resistant *Enterobacteriaceae* (Table 3) was higher in *E. coli* 92 (86.0%), followed by *K. pneumoniae* with 83 (60.1%). These were found to be resistant to less than two antibacterial agents.

According to the spectrum of action of the commercial produced antimicrobial agent, Figure 1 shows that Ciprofloxacin was sensitive to 89% of the *Enterobacteriaceae* followed by chloramphenicol 87%, ofloxacin 73%, Erythromycin 66%, gentamycin 63%, and Augmentin 57% while Septrin is resistant to 91% of the isolate followed Ampiclox 83%, sparfloxacin 73% and pefloxacin 67% of the *Enterobacteriaceae*.

The susceptibility profile of the cephalosporin tested (Table 4) shows that the synergic combination of Amoxicillin and clavulanic acid were much more effective and susceptible to 88% of the *K. pneumoniae* isolate, followed by Amoxicillin with 65% sensitivity. Ceftriaxone resisted to 92% of the *K. pneumoniae,* then cefotaxime 79%, and Cefuroxime 77% resistant to the test organism as shown in Figure 2.

From Figure 2, a total of 138 *K. pneumoniae* isolate were tested for ESBL production, 56 (40.60%) were producers while 82 (59.40%) were non-ESBL producers. All the ESBL producers resist Ceftriaxone and Cefuroxime, some were found to be resistant to amoxicillin while pure are susceptible. Non-ESBL producers are sensitive to the tested cephalosporins.

4. Discussion

Most of the clinically isolated enteric bacteria might be the main causes of nosocomial infection in the hospital setting likewise community-acquired infections; *Klebsiella* and *E. coli* have dominated almost every habitat (Chander, 2013). These contributed to the presence of the *Enterobacteriaceae* in the samples of the working population (26–55 years) and the aged (Sompolinsky, 2005). Urinary tract infection (UTI) and other congenital abnormalities that are related to a female patient, increase the rate at which the enteric bacteria were isolated from female patients 245 (50.5%). Recently there was an increase in the number of female patients attending hospital than the male patient (Kader, 2005).

Ciprofloxacin was the most sensitive antibiotic with 89% susceptibility against the other antibiotics. It was effective in the treatment of UTI, septicemia, and colitis as reported by Kyabaggu (2007), Bradford (2001) and (Knothe, 2013). Its effectiveness in the treatment

Table 2. Distribution of isolate by gender.

S/no	Isolate	Male *n* (%)	Female *n* (%)	Total (%)
1.	*K. pneumoniae*	41 (8.5)	97 (20)	138 (28.5%)
2.	*E. coli*	73 (15.0)	34 (7.0)	107 (22%)
3.	*Proteus* species	27 (5.6)	16 (3.3)	43 (8.9%)
4.	*Enterobacter* species	16 (3.3)	17 (3.5)	33 (6.8%)
5.	*S. typhi*	44 (9.1)	43 (8.9)	87 (18%)
6.	*Shigella* species	39 (8.0)	38 (7.8)	77 (15.8%)
Total	485	240 (49.5)	245 (50.5)	485 (100%)

Table 3. Frequency of the highly resistant *Enterobacteriaceae* (HRE).

S/no	Organism	Total number	HRE strain (%)
1.	*K. pneumoniae*	138	83 (60.1)
2.	*E. coli*	107	92 (86.0)
3.	*Proteus* species	43	27 (62.7)
4.	*Enterobacter* species	33	21 (63.6)
5.	*S. typhi*	87	67 (77.0)
6.	*Shigella* species	77	53 (68.8)
Total	485	485	343 (70.7)

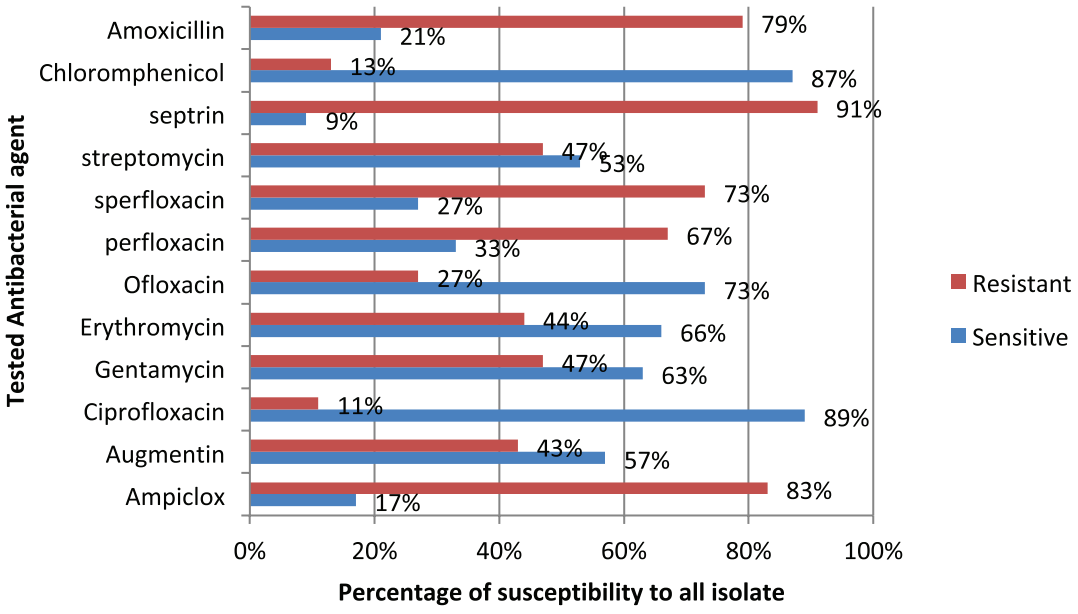

Figure 1. Antibacterial susceptibility profile of *Enterobacteriaceae* isolate.

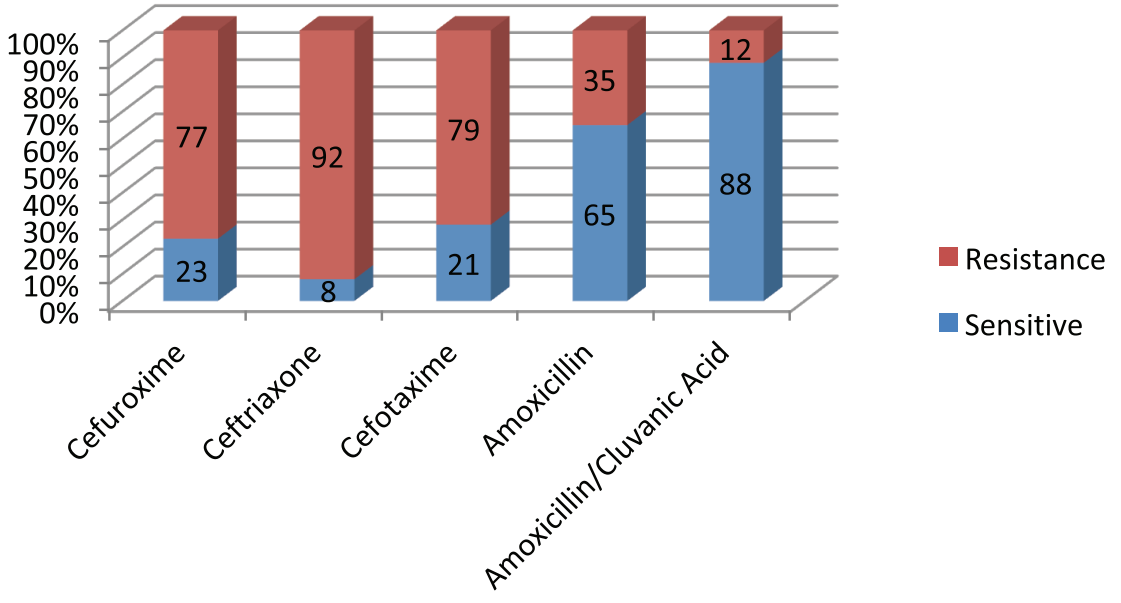

Figure 2. Susceptibility profile of cephalosporin's on *Klebsiella pneumonia.*

of many minor infections which might be treated with narrow-spectrum antibiotics and inappropriate use of the drug may cause many bacterial strains to develop resistance to ciprofloxacin, leaving it significantly less effective than it would have been otherwise (Vatopoulos and Kalapothaki, 1997). In this study ciprofloxacin has shown significant resistance to *E. coli*, these may be as a result of the recurrent prescription of Ciprofloxacin for the treatment of infections related to *E. coli*. The fact that ciprofloxacin is broad-spectrum antibiotics work by inhibiting both DNA gyrase, type II topoisomerase, and topoisomerase IV (Pommier, 2010); it resistant may be on the gene that code for topoisomerase II by the bacteria. Chloramphenicol was the second most susceptible drug with 87% sensitivity. Bacteria resistant to chloramphenicol must have to develop reduced membrane permeability or induces mutation at 50S ribosomal subunit, these have made chloramphenicol resistant much rear in community-acquired infection than nosocomial infection. Our

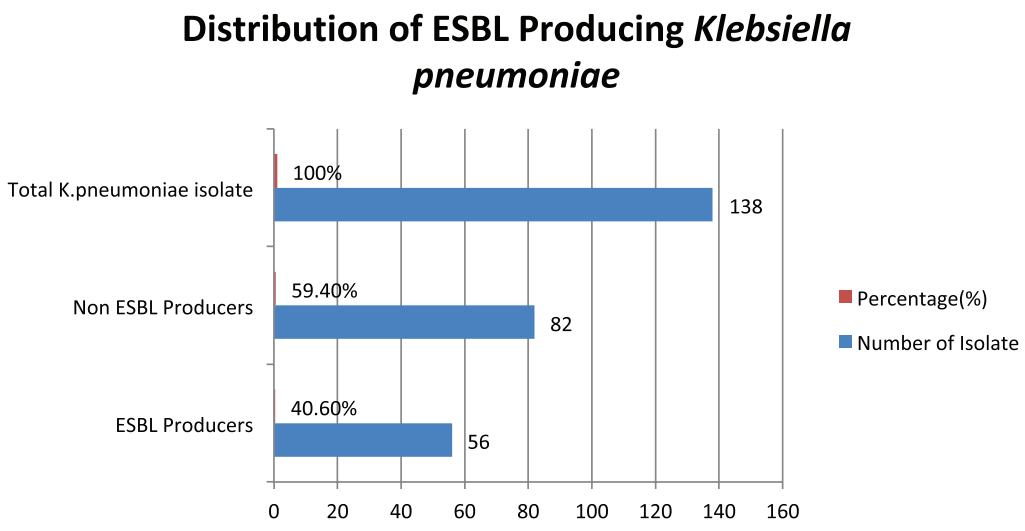

Figure 3. Distribution of ESBL Producing *Klebsiella pneumonia.*

study has shown the effectiveness of chloramphenicol on *Enterobacteriaceae*.

Septrin was resistant to 91% of the isolate; Trimethoprim and sulfamethoxazole as a component of the drug work efficiently combination to interfere with the generation of folic acid. (Widdowson, 1999) and Paolino (2004). Resistance to Septrin among isolates of *E. coli* varies greatly, from 10% to 70% in different parts of the world (Mukherjee, 2013). Strains isolated in the developing world are more often resistant than are strains isolated in Nigeria countries (Huovinen et al., 1995). In these parts of the world resistant to Septrin might have developed recurrent use of Septrin in the treatment of typhoid fever and other septicemia infections that are mostly caused by *Enterobacteriaceae* (Chaudhary, 2004). The 83% isolate was also resistant to Ampiclox which is a combination of Ampicillin and cloxacillin that comes from penicillin; resistance to penicillin develops from the capability of the bacteria to produce Betalactameses.

We have recorded 40.60% of ESBL producing *K. pneumoniae* among the 138 isolates tested which agreed with the study of (Vatopoulos and Kalapothaki, 1997). The high rate of ESBLs among hospitalized patients is a global problem. It is generally thought that patients infected by an ESBL-producing organism are at an increased risk of treatment failure with an expanded-spectrum beta-lactam. The prevalence of ESBL producing isolates of *K. pneumonia* varies in different countries (Sompolinsky, 2005). In conclusion, the spread of *Enterobacteriaceae* among clinical isolate is of concern as it has been the part of the etiological agent for most domestic infection. The prevalence of *K. pneumoniae* within the family of *Enterobacteriaceae* in the clinical isolate is a justification of the spreading nosocomial infection and the growing percentage of urinary and respiratory infection in the community (Widdowson, 1999). A synergic combination of generations cephalosporins should be considered in the treatment of *Klebsiella* infection as the increase in ESBL producing *Klebsiella pneumoniea* is eminent. A combination of Amoxicillin and clavulanic acid was proven effective in this study in the treatment of ESBL producing strains.

Acknowledgments

This work would not have been successful without the effort of Reemee medicare management and laboratory staff especially Muhammad Sabiu who help me during sample collection and other bench work. So must I also acknowledge Mal. Yunusa of Bauchi state specialist hospital for support. This work is dedicated to my family and all those that suffered from infections by ESBL producing *K. pneumoniae.*

References

Amraie H, Shakib P, Rouhi S, Bakhshandeh N, Zamanzad B. Prevalence assessment of magA gene and antimicrobial susceptibility of Klebsiella pneumoniae isolated from clinical specimens in Shahrekord, Iran. Iranian J Microbiol 2014; 6(5):311–6.

Boucher TG. Bad bugs, no drugs: no ESKAPE! an update from the infectious diseases society of America. Clin Infect Dis 2009; 48:1–12.

Bradford, PA. (2001). Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology,

and detection of this important resistance threat. Clin Microbiol Rev 2001; 14:933–51.

Chander SC. (2013). Prevalence of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* urinary isolates in a tertiary care hospital in Kathmandu, Nepal. BMC Res Notes 2013; 6:487–92.

Chaudhary, A. R. (2004). Extended spectrum β-lactamases (ESBL) – an emerging threat to clinical therapeutics. Indian J Med Microbiol 2004; 22:75–80.

CLSI. Performance standards for antimicrobial susceptibility testing. CLSI, M100-S25, Wayne, PA, 2015.

Cohen DC. Rapid detection of TEM, SHV and CTX-M extended-spectrum β-lactamases in *Enterobacteriaceae* using ligation-mediated amplification with microarray analysis. J Antimicrob C 2012; 34–154.

Huovinen P, Sundström L, Swedberg G, Sköld O. Trimethoprim and sulfonamide resistance. Antimicrob Agents Chemother 1995; 39(2):279–89; doi: 10.1128/AAC.39.2.279. PMID: 7726483; PMCID: PMC162528.

Kader AK. Extended-spectrum beta-lactamases in urinary isolates of *Escherichia coli*, *Klebsiella pneumoniae* and other gram-negative bacteria in a hospital in Eastern Province. Saudi Arabia. Saudi Med J 2005; 26:956–9.

Kattel HP, Acharya J, Mishra, SK. (2008). Bacteriology of urinary tract infection among patients attending Tribhuvan University Teaching Hospital, Kathmandu, Nepal. J Nepal Assoc Med Lab Sci 2008; 9:25–9.

Knothe SP. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and Serratia marcescens. Infection 2013; 11:315–7.

Kyabaggu DE. The sensitivities to first-line antibiotic therapy of the common urinary tract bacterial infections detected in urine samples at a hospital in metropolitan Kampala (Uganda). Afr Health Sci 2007; 7(4):214–2.

Mukherjee BS. Multidrug-resistance and extended spectrum beta-lactamase production in uropathogenic *E. coli* which were isolated from hospitalized patients in Kolkata. India J Clin Diagn Res 2013; 7:449–53.

Paolino ED. *In vitro* activity of colistin against multidrug-resistant Gram-negative bacteria isolated at a major army hospital during the military campaigns in Iraq and Afghanistan. Clin Infect 2004; 45:140–1.

Paterson BR. Extended-spectrum -lactamases: a clinical update. Clin Microbiol Rev 2005; 18, 657–86.

Pommier L. E. (2010). DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. *Chem Biol 17*(5), 421–33. doi:10.1016/j.chembiol.2010.04.012

Sahuquillo-Arce PHQ. Approach to urinary ESBL-producing *Escherichia coli*. J Pharmacovigilance. 2014; 2:133.

SNCCL. Performance standards for antimicrobial susceptibility testing. NCCLS, Atlanta, GA, 2005.

Sompolinsky NY. Integron-mediated ESBL resistance in rare serotypes of *Escherichia coli* causing infections in an elderly population of Israel. J Antimicrob Chemother 2005; 55:119–22.

Vatopoulos A, Kalapothaki V. Bacterial resistance to ciprofloxacin in Greece: results from the national electronic surveillance system (PDF). Emerg Infect Dis 1997; 5(3):471–6.

Widdowson CA. Molecular mechanisms of resistance to commonly used non-betalactam drugs in *Streptococcus pneumoniae*. Semin Respir Infect 1999; 14:255–68.