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ANTIBIOTIC SUSCEPTIBILITY PATTERN AND PHENOTYPIC CHARACTERIZATION OF EXTENDED-SPECTRUM-BETA-LACTAMASE-PRODUCING *Enterobacteriaceae* ISOLATED FROM VARIOUS CLINICAL SAMPLES

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Abstract

The spread of antibiotic resistant organisms and those producing extended spectrum β-lactamases (ESBL) has become a health care problem worldwide in communities and hospitals, as it leads to more complicated infections, longer duration of treatment, and increases in patient mortality. In the present study, we analyzed 226 clinical samples in order to assess the spread of ESBL-producing *Enterobacteriaceae*. 196 *Enterobacteriaceae* were identified and classified as members of the genera *Pantoea*, *Klebsiella*, *Escherichia*, *Enterobacter*, *Serratia*, *Proteus*, *Citrobacter*, and *Raoultella*. The results of susceptibility testing of isolated strains to 19 antibiotics showed that the most part of the isolates were highly resistant ($p<0.01$) to the tested β-lactams: penicillins and penicillin like antibiotics (amoxicillin, ticarcillin and amoxicillin-clavulanic acid), first-generation cephalosporins (cephalexin), and second generation cephalosporins (cefoxitin). 60.20% of the *Enterobacteriaceae* isolates were multi-drug resistant (MDR) strains. Resistant isolates to third generation cephalosporins were tested for ESBL by tree methods, concluding its presence in 29.59% of the isolates by double-disk synergy test, 27.55% by the disk approximation method and by 31.63% double-disk test. High levels of MDR strains and ESBL- producing *Enterobacteriaceae* in our study suggest the need for applying specific infection control measures, and rational antibiotic use to reduce the selection pressure and prevent dissemination of resistant bacteria.

Keywords: antimicrobial susceptibility, clinical samples, *Enterobacteriaceae,* extended-spectrum β-lactamases (ESBL)- producing and multi-drug resistance.

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1. Introduction

Over past few decades, antibiotic resistance of microorganisms has become a health care problem worldwide in communities and hospitals, a great deal has happened and many kinds of research have started aimed to determine the cause of the rapid spread of multidrug-resistant bacteria (Pereckaite et al., 2018). Despite many national and international reports, including that of the World Health Organization, urging ways to curtail it, the problem continues to grow and to evolve from one decade to the next (Munita and Arias, 2016). The recent report showed that resistance to antibiotics drugs can arise through various mechanisms, including target modification (expression of alternative penicillin-binding proteins), reduction in cell permeability through porin modification and efflux pump expression, and

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production of modified enzymes (Tooke et al., 2019).

The emergence of multidrug-resistant bacteria and specially, ESBL- producing *Enterobacteriaceae* has become a worldwide concern. These bacteria create a therapeutic challenge in daily management of infectious diseases due to their resistance to additional classes of antibiotics reduce switching to an alternative antimicrobial regimen (Kharrat et al., 2018). The extended spectrum β-lactamase**s** (ESBL) belong to the group of enzymes which have the ability to hydrolyze the β-lactam ring in the structure of various types of newer β-lactam antibiotics including the extended-spectrum (or third-generation) cephalosporins and monobactams (aztreonam) but not the cephamycins (cefoxitin and cefotetan) and carbapenems (Kunishima et al., 2019).

Moreover, the functional classification scheme suggests the subdivision of β-lactamases to: cephalosporinases, serine β-lactamases and Metallo-β- lactamases (MBLs) (Rood and Li, 2017). It has been reported that the species which produce ESBLs are mostly *Klebsiella* species and *Escherichia coli.* Moreover, at a reduced frequency these enzymes are now found in many different species of other members of *Enterobacteriaceae* family, such as *Proteus* spp., *Enterobacter* spp., *Citrobacter* spp., *Serratia marcescens* and *Morganella* (Muthupandian et al., 2018).

Over the last 30 years, various phenotypic methods have been proposed to detect or confirm ESBL production by *Enterobacteriaceae* (Donaldson et al., 2008; Garrec et al., 2011; Jarlier et al., 1988). Nevertheless, all methods utilize the two crucial characteristics of ESBL-producing bacteria: reduction of susceptibility to extended-spectrum cephalosporins and inhibition of the enzymes β-lactamases activity by clavulanic acid (Tsering et al., 2009). The Clinical Laboratory Standards Institute (CLSI) recommends phenotypic screening and confirmatory tests to identify the ESBL producers (Garrec et al., 2011). Many reports proposed screening strains based on decreased susceptibility to extended-spectrum cephalosporins in primary susceptibility testing and to use the confirmatory tests of ESBL production.

The objective of this study is to determine the prevalence of ESBL-producing *Enterobacteriaceae* among strains isolated from clinical samples as well as the prevalence of multi-drug resistant strains.

2. Material and methods

2.1. Collection of strains

A total of 226 clinical specimens including blood culture, urinary catheters, pus, wound swab, and tracheal aspirate were collected during the period of 2018 to 2019, at Tebessa hospital. In this hospital, approximately 15000 patients are admitted at the outpatient department per year and more than 900 operations and invasive diagnostic therapeutic procedures are performed annually.

The samples of pus and wound were taken by swab, followed by enrichment on nutrient broth. For urinary catheter samples, enrichment is done by inoculating 1 mL of urine in 5 mL of nutrient broth. For blood culture, the blood sample is incubated for 24 hours at 37°C, then isolation, as for the other samples, is carried out on Mac Conkey agar according to the streak method.

All isolates were analyzed both by conventional bacteriological methods and by Mini-API, a semi-automatized assay (bioMérieux, Marcy l'Etoile, France). Isolates were frozen at -30°C in brain-heart infusion broth with 15% glycerol until processed for further experimentation.

2.2. Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed on Mueller-Hinton agar (MH; BioMérieux, Marcy-l'Étoile, France) by standard disk diffusion method, using disk antibiotics (Liofilmchem) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2018) guidelines. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 strains were used as quality controls for antimicrobial susceptibility and the ESBL screening tests, respectively. Isolates that exhibited zone diameters of ≤ 22 mm for ceftazidime, ≤ 25 mm for ceftriaxone, and ≤ 27 mm for cefotaxime and aztreonam were submitted to the ESBL detection tests.

Screening of multi-drug resistant (MDR) strains

MDR strains were screened according to the definition of Magiorakos et al. (2012): if the isolates were resistant to representative antibiotics of at least three different classes of antimicrobial agents, they were regarded as multi-drug resistant strains.

2.3. ESBL detection

2.3.1. Double-disk synergy test (DDST)

The double-disk synergy test was carried out according to Jarlier et al. (1988). Third-generation cephalosporin disks, cefotaxime (CTX 30 µg), ceftazidime (CAZ 30 µg), ceftriaxone (CRO 30 µg), or aztreonam disk (ATM 30 µg) was placed 30 mm (center to center) from a central disk containing amoxicillin/clavulanic acid (AMC 20/10 µg). The ESBL production is suspected when the inhibition zone around any of the four antibiotic disks was enhanced on the side of the disk containing clavulanic acid, resulting in a characteristically shaped zone referred to as a "champagne-cork," "keyhole," "ellipsis," or "phantom image".

2.3.2. Disk approximation method (DAM)

This test was conducted as described by Rahal (1999). Cefotaxime (30 µg) disks were placed at 15, 20, 25, and 30 mm center to center away from an AMC disk which is placed in the center of the plate. The test was considered positive if there is restoration of cefotaxime activity resulting by the appearance of synergy image between CTX and AMC.

2.3.3. Double-disk test (DDT)

This test was performed as described by Rahal, (2005). Disks of amoxicillin/clavulanic acid (AMC 20/10 µg) and third-generation cephalosporins (CTX 30 µg) were placed at 25 mm (center to center) on Mueller Hinton agar inoculated with the tested strain. After 1 h of incubation, the AMC disk was replaced by CTX disk. The test was considered positive for ESBL production if the inhibition diameter of CTX disk applied after pre-diffusion of the AMC disk is ≥5 mm with respect to the diameter of CTX disk.

2.4. Statistical analysis

To determine if there is a statistically significant difference between the various parameters, *t*-test was performed. The differences were considered significant at $p < 0.05$. The Pearson's correlation analysis was performed to find out relationships among various characteristics.

3. Results and discussion

Extended spectrum ß-lactam antimicrobial drugs are commonly included in empirical antibiotic regimens for treatment of Gram-negative sepsis but the emergence of ESBL producing bacteria have become a major public health concern. β-lactamases enzymes are most often encoded either by the chromosomal genes or by the transferable genes which can easily be transferred between isolates. Initially, these enzymes were commonly found in a variety of *Enterobacteriaceae* species; and the majority of the ESBL producing strains are *Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli, Enterobacter, Citrobacter, Proteus, Morganella morganii, Serratia marsescens,* and other Gram-negative bacilli like *Pseudomonas aeruginosa,* and *Burkholderia cepacia* (Tsering et al., 2009).

Since the begin of the millennium, particular attention is paid to the emergence of Gram negative bacterial species with acquired resistance to various broad spectrumβ-lactams and other classes of antimicrobials (Kuralayanapalya et al., 2019). This emphasizes the demands on routine Clinical Microbiology laboratory to investigate the potential of MDR and ESBL production on every suspected isolates. The present study has examined the prevalence of *Enterobacteriaceae* strains from clinical specimens, their antibiotic-susceptibility pattern, and production of ESBL among MDR strains with phenotypic approaches, giving a first insight into the occurrence of these isolates in the country.

3.1. Distribution of bacterial species with sample source

In the present study, a total of 400 Gram negative bacilli representing different colony morphologies were recovered from 226 clinical specimens including urinary catheters, pus, blood culture, tracheal aspirate and wound swab, from both inpatients and outpatients of all age groups. Among these, 196 isolates were identified as members of *Enterobacteriaceae* family. The distribution of the isolates from various samples is presented in Table 1.

The prevalence of *Enterobacteriaceae* bacteria in various clinical specimens was 36.2%, while prevalence of multidrug resistance was 60.20%. Similar rate was also reported by Magiorakos et al. (2012).

According to the distribution of strains in terms of species, the most abundant species was *Klebsiella pneumoniae*, which was the most prevalent with 44 (22.45%) isolates followed by *E. coli* with 30 (15.31%), *Enterobacter cloacae* with 22 (11.22%) isolates, *Serratia ficaria* with 20 (10.20%), *Pantoea* spp with 18 (9.18%), *Serratia marcescens* with 16 (8.16%), *Klebsiella oxytoca* with 13 (06.63%), and *Citrobacter freundii* with 12 (6.12%) isolates. In addition, other pathogenic genera have been isolated, but with lower frequencies: *Proteus vulgaris* 06 (3.06%) and *Raoultella ornithinolytica* with 07 (03.57%).

The distribution of the isolates from various clinical specimens has showed that urine was the source of 74/196 (37.75%) of the isolates, indicating that urinary catheters are one of the most common infections worldwide. Blood culture 33/196 (16.84%) was the second major source of isolates reflecting the relatively high frequency of *Enterobacteriaceae* involved in bacteremias. The rest of the isolates collected in our study were from pus, tracheal aspirate and wound swab. The agents responsible for urinary tract infections are mainly enterobacteria which are part of the normal faecal flora, peri-urethral colonization same to be a necessary stage for the occurrence of the infection. However, for hemocultures, enterobacteria are least common because there are other agents which arise from other entry points other than the digestive tract corresponding to enterobacteria. The frequency of specimen distribution in the present study reflects the prevalence of *Enterobacteriaceae* in similar settings reported elsewhere (Nepal et al., 2017; Tacconelli et al., 2019).

3.2. Phenotypes of antibiotic resistance

Antibiotics sensitivity pattern amongst *Enterobacteriaceae* isolates (Table 2) revealed high frequency of resistance to β-lactams ($p<0.01$): amoxicillin (AMX) 95.41%, ticarcillin (TIC) 94.39%, amoxicillin/clavulanic acid (AMC) 91.33%, cephalexin (CL) 78.06%, cefalotin (KF) 67.86%, cefotaxime (CTX) 78.57%, ceftazidime (CAZ) 72.96%, aztreonam (ATM) 76.53%, and cefoxitin (FOX) 67.35%. Regarding non-β-lactams antibiotics, resistance rates were high for fosfomycin (FOS) 64.79% and nalidixic acid (NA) 55.61%; significant for cotrimoxazole (COT) 46.43% and ciprofloxacin (CIP) 43.88%; moderate for tobramycin (TOB) 36.73%, nitrofurantoin (F) 28.06%, and low for amikacin (AK)18.37%, chloramphenicol (C) 17.86%,

gentamicin (GM) 14.28%. Only imipenem (IPM) was effective against more than 94% of the isolates. Consistent with previous report (Jena et al., 2018; Nakai et al., 2016), our study revealed that *Enterobacteriaceae* isolates have various antimicrobial susceptibility profiles. The high β-lactam resistance might be explained by its intensive use and the selective pressure, which accelerated the emergence of resistance to β-lactamase inhibitor combinations. Similar with previous reports, imipenem seem to be appropriate for the empirical treatment of ESBL infection, because of its very high rate of susceptibility Jena et al., 2018). However, our data suggested that resistance against aminoglycosides was much reduced (18.37% against AK and 14.28% GM). Hence, aminoglycosides could also be used cautiously as antimicrobial empiric therapy (Nakai et al., 2016).

Table 1. Distribution of *Enterobacteriaceae* isolates and ESBL-producing strains from different samples

	Urinary catheters		***Pus***		***Blood culture***		***Tracheal aspirate***		***Wounds swab***		***Total***	
Bacterial species	***No. of isolates***	***No. of ESBL-producing isolates (%)***	***No. of isolates***	***No. of ESBL-producing isolates (%)***	***No. of isolates***	***No. of ESBL-producing isolates (%)***	***No. of isolates***	***No. of ESBL-producing isolates (%)***	***No. of isolates***	***No. of ESBL-producing isolates (%)***	***No. of isolates***	***No. of ESBL-producing isolates (%)***
Klebsiella pneumoniae (N= 44)	12	08	00	00	10	02	22	08	00	01	44	19
Klebsiella oxytoca (N= 13)	07	02	00	00	04	01	02	01	00	00	13	04
Klebsiella ornithinolyitica (N= 08)	02	01	00	00	00	00	00	00	06	02	08	03
E. coli (N= 30)	12	05	06	00	07	00	00	00	05	01	30	06
Pantoeaspp (N= 18)	04	01	00	00	03	00	04	01	07	03	18	05
Enterobacter cloacae (N= 22)	10	05	04	01	04	02	03	02	01	00	22	10
Serratia fecaria (N= 20)	08	03	05	01	02	01	05	00	00	01	20	06
Serratia marcescens (N= 16)	05	02	06	01	01	00	02	00	02	00	16	03
Proteus vulgaris (N= 06)	04	01	00	01	00	00	00	00	02	00	06	02
Citrobacter freundii (N= 12)	06	02	01	02	01	00	04	00	00	00	12	04
Raoultella ornithinolyitica (N= 07)	04	01	02	01	01	00	00	00	00	00	07	02
Total (%)	74	31 (41.89)	24	07 (29.17)	33	06 (18.18)	42	12 (28.57)	23	08 (34.78)	196	64 (32.65)

Table 2. Antimicrobial resistance of *Enterobacteriaceae* isolates

Resistance phenotypes rates																		
AMC	***AMX***	***TIC***	***CL***	***KF***	***CTX***	***CAZ***	***ATM***	***IPM***	***FOX***	***C***	***AK***	***GM***	***TOB***	***FOS***	***NA***	***CIP***	***F***	***COT***
40	42	40	35	30	37	33	29	02	30	07	10	04	16	27	22	23	12	25
12	12	10	11	11	12	09	13	00	11	03	04	06	07	09	08	07	10	07
08	08	08	07	08	06	04	08	00	04	01	00	00	02	04	06	04	00	04
25	29	28	22	16	23	20	18	04	20	08	10	05	13	25	15	14	10	11
18	18	18	14	11	10	14	14	01	11	00	05	06	04	10	10	06	05	06
20	22	22	15	20	21	18	18	00	14	07	02	05	07	15	16	07	08	10
20	18	18	20	13	14	15	15	01	16	04	00	02	08	16	08	10	06	10
15	16	12	10	09	13	14	10	03	12	01	00	00	05	11	10	05	03	07
04	04	06	06	06	04	03	06	00	01	02	00	00	01	00	02	02	00	02
10	11	12	08	07	10	11	12	00	10	00	04	00	04	09	09	04	01	6
07	07	06	05	02	04	02	07	00	03	02	01	00	04	01	03	04	00	03
179 (91.33)	187 (95.41)	180 (94.39)	153 (78.06)	133 (67.86)	154 (78.57)	143 (72.96)	150 (76.53)	11 (05.55)	132 (67.35)	35 (17.86)	36 (18.37)	28 (14.28)	72 (36.73)	127 (64.79)	109 (55.61)	86 (43.88)	55 (28.06)	91 (46.43)

AMC: amoxicillin/ clavulanic acid, AMX: amoxicillin, TIC: ticarcillin, CL: cephalexin, KF: cefalotin, CTX: cefotaxime, CAZ: ceftazidime, ATM: aztreonam, IPM: imipenem, FOX: cefoxitin, C: chloramphenicol, AK: amikacin, GM: gentamicin, TOB: tobramycin, FOS: fosfomycin, NA: nalidixic acid, CIP: ciprofloxacin, F: nitrofurantoin, COT: cotrimoxazole

3.3. Prevalence of ESBL production

Several phenotypic methods have been proposed for the detection of ESBLs in clinical isolates (Cormican et al., 1996; Rood and Li, 2017; Wiegand et al., 2007). These tests should accurately discriminate between bacteria producing these enzymes and those with other mechanisms of resistance to β-lactams, e.g., broad spectrum β-lactamases, inhibitor-resistant β-lactamases and cephalosporinase overproduction. In the present study we have used three phenotypic detection tests, based on the synergy between a third-generation cephalosporin and clavulanate: the double-disk synergy test, double-disk test and the disk approximation method. Among the 196 *Enterobacteriaceae* isolated strains, 118 (60.20%) were screened as MDR and highest frequency was found to be *Klebsiella pneumoniae* 31 (70.45%). Nevertheless, 62 (31.63%) were defined as ESBL producers (Table 3). The DDST suspected the presence of ESBL in 58 (29.59%) strains, and the disk approximation method (DAM) was positive for 54 (27.55%) isolates, whereas the double-disk test (DDT) confirmed the presence of ESBLs in 62 (31.63%) strains (Fig. 1). The application of the double disc synergy tests that combine amoxicillin-clavulanate with cefepime, same to be the most appropriate method for detection of the ESBL producing strains because it can clearly demonstrate the inhibition of ESBLs by clavulanic acid. In recent years, many study have reported that the prevalence of ESBL producers varies across continents and countries and also within hospitals and these difference may be due to geographical variation, the diagnostic methods and the difference in antibiotic practices of the study area (Jamali et al., 2017; Sun et al., 2014; Tschudin-Sutter et al., 2017). The overall data on ESBL-producing *Enterobacteriaceae* in the North African countries are extremely worrisome and this region might indeed be one of the major epicenters of the global ESBL pandemic. In Algeria, data indicates the prevalence of ESBL's producer *Enterobacteriacae* ranges were found between 16.4 and 99% of which Class A ESBLs were most common and exhibits the encoding plasmid of AmpC (pAmpC) was present (Meradi et al., 2011; Nedjai et al., 2012; Touati et al., 2012). In Egypt, The ESBL *Enterobactericeae* prevalence among clinical strains varies between 11 and 42.9% both in hospital and community samples by which the most frequently detected beta-lactamase gene was CTX-M (Fam et al., 2011).

The predominant ESBL type in Tunisia were found to be class A and D ESBLs, pAmpC, and carbapenemases, and their prevalence ranges from 11.7 to 77.8% causing both community acquired and hospital acquired infections (Elhani et al., 2010; Kharrat et al., 2018). In Morocco, low prevalence rates between 1.3 and 7.5% have been found, of which CTX-M genes was the most prevalent in this area (Bourjilat et al., 2011; El bouamri et al., 2015). A recent investigation conducted in Libya showed that the prevalence of ESBL are found between 6.7 and 32.6% in hospital samples and 13.4% in community samples (Ahmed et al., 2014).

The prevalence of ESBL production among 05 representative clinical specimens by species showed that 19 of 44 *K. pneumoniae*, 06 of 30 *E. coli* isolates, 10 of 22 *Enterobacter cloacae*, 06 of 20 *Serratia ficaria,* 05 of 18 *Pantoea spp,* 03of 16 *Serratia marcescens,* 04 of 15 *Klebsiella oxytoca,* 04 of 12 *Citrobacter freundii,* 04 of 15 *Klebsiella oxytoca,* 03 of 08 *Klebsiella ornithinolyitica* and 02 of 07 *Raoultella ornithinolytica* were ESBL producers.

Table 3. Phenotypic detection of ESBL-producing *Enterobacteriaceae*

		ESBL- producing by						***MDR strains***
		Double-disk synergy test (DDST)		***Disk approximation method (DAM)***		***Double disk test (DDT)***		
Species	***No. of Isolates (N=196)***	***Positive (N =58)***	***negative (N=138)***	***Positive (N=54)***	***negative (N=142)***	***Positive (N=62)***	***negative (N=134)***	***No. (%)***
Klebsiella pneumoniae	44	19	30	12	32	13	31	31 (70.45)
Klebsiella oxytoca	13	04	09	04	09	04	09	08 (61.54)
Klebsiella ornithinolyitica	08	02	06	02	06	02	06	04 (50.00)
E. coli	30	07	23	09	21	09	21	16 (53.33)
Pantoea spp	18	04	14	04	14	05	13	08 (44.44)
Enterobacter cloacae	22	10	12	07	15	10	12	18 (63.64)
Serratia ficaria	20	07	13	09	11	06	14	09 (45.00)
Serratia marcescens	16	03	13	03	13	05	11	10 (62.50)
Proteus vulgaris	06	02	04	00	06	02	04	03 (50.00)
Citrobacter freundii	12	04	08	04	08	04	08	07 (58.33)
Raoultella ornithinolytica	07	01	06	00	07	02	05	04 (57.14)
Total (%)	196 (100)	58 (29.59)	138 (70.41)	54 (27.55)	142 (72.45)	62 (31.63)	134 (68.36)	118 (60.20)

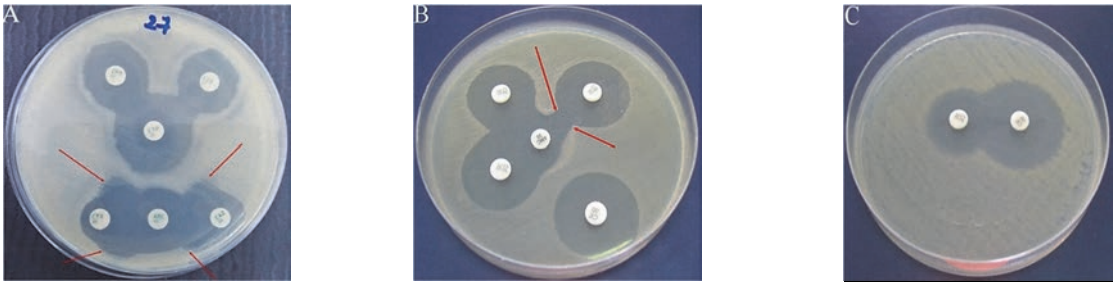

Fig.1. Example showing phenotypic detection of ESBLs producer by (A), double disk synergy test (DDST). (B), positive Disk approximation method (DAM). (C), confirmed ESBLs producer by Double disk test (DDT)

There were differences in the number of resistant strains isolated from different specimens, because infections due to ESBLs- producing *Enterobacteriacea* depend on several risk factors including: repetitive urinary tract infections, underlying pathologies, prior antibiotic therapy (cephalosporins and fluoroquinolones), prior hospitalization, duration of hospitalization, ICU length of stay (incubation catheterization, mechanical ventilation). For septicemia, two or three antibiotics are initially administered jointly, especially when their origin is unknown. Then, when the test results are available, the doctor can adapt the treatment using the most effective antibiotic against the specific bacteria causing the infection. For urinary infections, cephalosporins, fluoroquinolones and cotrimoxazole. For respiratory infections, beta-lactams and fluoroquinolons are used. For wound infections, beta-lactams aminoglycosids and fluoroquinolones are used.

Our finding has demonstrated that from the overall group of ESBL, *Klebsiella pneumoniae* was the most frequently identified strain among ESBL producers. This percentage is similar to prevalence of ESBL production worldwide among this species when compared with the (53.3%) in Ethiopia (Moges et al., 2019), and 39.5% in Mexico (Silva-Sanchez et al., 2011).

By clinical specimens, 31 (41.89%) of 74 urinary catheters isolates, 12 (28.57%) of 42 tracheal aspirate isolates, 06 (18.18%) of 33 blood culture isolates, 07 (29.17%) of 24 pus isolates and 08 (34.78%) of 23 wound swab isolates were ESBL producers. Compared to our finding, multiple studies have clearly demonstrated that urinary tract infections (UTIs) caused by ESBL producing bacteria are a very common healthcare issue and represent the second most common type of infection in humans (Castillo-Tokumoria et al., 2017; Xu and He, 2019). This observation supports the findings of Jagdeesh et al. (2014) who reported among screen positive isolates for ESBL, 45.1%, 46.7% and 29.4% ESBL producers from urine, exudates/pus and sputum respectively, while 100% ESBL producers were detected in stool. However, a study by Sharma et al. (2013) recorded that respiratory tract samples (63.83%) was the major source of ESBL-producing strains followed by stool samples, urine, body fluid, pus, and blood.

4. Conclusions

Infections caused by ESBL-producing *Enterobacteriaceae* have dramatically increased worldwide, and this "evolving crisis" is recently considered as one of the most important public health threats.

Our study revealed that MDR isolates in the present study was 60.20%. Among phenotypically tested *Enterobacteriaceae,* 31.63% were ESBL producers. The rate of isolation of ESBL Gram-negative bacteria in the present study is very serious issue, which suggests the dissemination of ESBL producing isolates in hospitals.

We emphasize that more studies should be carried out to address the problem of healthcare-associated infections caused by ESBL-producing bacteria, especially in Algeria, where antibiotic abuse and irrational use is a common practice.

The continuous monitoring and careful selection usage of antibiotics substance, periodic surveillance of multidrug resistance patterns and efforts to decrease empirical antibiotic therapy would be greatly necessary step in addressing the problems associated with the spread of ESBLs.

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