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Phenotypic Characterization of Non-Beta Lactam Multidrug Resistance in Extended-Spectrum Beta-Lactamase Producer and Non-Producer Enterobacteriaceae

Cakir N^{1*}, Süer K¹, Özdemir SB¹, Güler E¹ and Etikan I²

¹Department of Infectious Disease and Clinical Microbiology, Faculty of Medicine, Near East University, Nicosia, Northern Cyprus, TRNC Mersin 10, Turkey

²Department of Biostatistics, Faculty of Medicine, Near East University, Nicosia, Northern Cyprus, TRNC Mersin 10, Turkey

*Corresponding author: Cakir N, Department of Infectious Disease and Clinical Microbiology, Faculty of Medicine, Near East University, Nicosia, Northern Cyprus, TRNC Mersin 10, Turkey; Tel: +90548847197; E-mail: nedim.cakir@neu.edu.tr

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Abstract

Purpose: The aim of this study is to detect the correlations between ESBL-producing, and the pattern, and extensiveness of MDR of other common non-beta-lactam antibiotics in Enterobacteriaceae strains.

Methods: A total of 170 ESBL-producer strains and the same amount of ESBL-non-producer Enterobacteriaceae strains were collected as study and control groups from our Clinical Microbiology Laboratory. Antibiotic susceptibility tests and ESBL detection were conducted on both microorganism groups by routine bacteriologic methods. The antibiotic susceptibilities have been listed on a strain basis, and they were classified according to the extensiveness of resistances.

Results: When comparing the ESBL-producer to the ESBL-nonproducer strains, higher individual resistance rates were detected to fluoroquinolones, trimethoprim/sulfamethoxazole, aminoglycosides, piperacillin/tazobactam, nitrofurantoin and carbapenems in various percentages (69.40% vs 23.50%; 68.20% vs 30%; 34.70% vs 5.30%; 27.20% vs 4.73%; 23.50% vs 1.90 and 18.90% vs 5.20% respectively).

The three to six-drug MDRs in the same isolate has been found in 36 different strains of ESBL-p, although no strains were detected in the control group (21% vs 0).

The three most prevalent triplet co-existences of MDR were sulfamethoxazole/trimethoprim+fluoroquinolones+aminoglycosides, and sulfamethoxazole/trimethoprim+fluoroquinolones+piperacillin/tazobactam, and sulfamethoxazole/trimethoprim+fluoroquinolones+carbapenem resistance combinations in both groups, but, their proportions were statistically higher in the study group (43 vs 4, 31 vs 3, and 22 vs 1 strains).

Conclusions: ESBL and carbapenemase-producing Enterobacteriaceae control the other wide range of non-beta-lactam antibiotic resistance in various combinations, according to our study.

Keywords: Multidrug resistance; Extended spectrum betalactamase; Co-resistance; Enterobacteriaceae

Introduction

Extended-spectrum beta-lactamase-producing (ESBL-p) Enterobacteriaceae strains may cause emergent infections, both in the community and in hospital settings. These enzymes may inactivate a wide-range of beta-lactam group antibiotics, including nearly all cephalosporins [1-3].

The other major problem in the treatment of infectious diseases is the presence of MDR in the wide range of microorganisms, including Enterobacteriaceae. These group resistances also, contribute to the emergence and limit the treatment options of these microorganism infections.

Magiorakos et al. categorized a group of microorganisms including Enterobacteriaceae according to their extensiveness of antibiotic resistance. In order to achieve this, they firstly classified all effective antibiotics into "categories" and they placed similar "antibiotic agents" into the same category. The authors classified bacterial resistance as: "Multidrug-resistant (MDR)"; "extensively drug-resistant (XDR)"; and "Pandrug resistant (PDR)". They classified 31 effective antimicrobial agents for Enterobacteriaceae within 17 categories, including all beta-lactam antibiotics, as well as non-beta-lactams, and ESBL-resistant antibiotics (ESBL-RA) such as carbapenems [4].

According to these definitions, ESBL-p strains are automatically involved within MDR.

Carbapenemases are relatively different enzymes and mainly control carbapenems as well as another beta-lactam

resistance [5]. Generally, they are carried on a series of plasmids such as OXA-48, and others. These genes can harbor some non-beta-lactam resistance genes, and they can move together[6,7].

This characteristics may explain these ESBL - non-beta-lactam co-resistances. The most prevalent harboring genes are fluoroquinolone, trimethoprim/sulfamethoxazole, and aminoglycoside resistant genes to ESBL's and/or carbapenemases.

The other possible mechanism of wide range of resistance is multidrug efflux pumps. These subcellular organelles are the expeller a broad range of antibiotics and, even some heavy metals- drive the acquisition of additional resistance mechanisms by lowering intracellular antibiotic and other compounds concentration [8].

One problem that still exists is how many resistance genes may harbor and are placed in genetic structures to move together in enteric isolates in clinical settings [9-12]. In daily medical practices, detailed information on the transferable co-resistance genes containing elements will be a practical guide to choose effective antibiotic treatment options.

The aim of this study is to detect any correlations between ESBL positivity and MDR amongst ESBL-RA in Enterobacteriaceae strains and to analyze the screening of the extensiveness and co-existence of these resistance.

Materials and Methods

Study-related definitions

Various definitions have been made to facilitate the explanation of this study, as follows:

ESBL-RA: Abbreviation of ESBL-resistant antibiotics. It consists all non-beta-lactam antibiotics, carbapenems, and piperacillin/tazobactam that is resistant to conventional ESBLs.

Individual resistance: Single resistance to ESBL-RA in MDR enteric isolates

Extensiveness of MDR: The number of resistances to ESBL-RA detected within the same isolate. They are defined as "one-drug MDR" to "seven-drug MDR", or more.

Co-existence of resistance: The resistance combinations to ESBL-RA within the same enteric isolates

This prospective, case-control observational study was implemented between March 2016 and September 2017 in our 150-bed tertiary care teaching hospital in Nicosia, Northern Cyprus. During the study period, a total of 170 ESBL-p Enterobacteriaceae strains were collected.

Inclusion and exclusion criteria

The repeated cultures were excluded. Members of the Enterobacteriaceae family other than *E. coli* and *Klebsiella* strains were also excluded, because of their proportion was significantly low.

Control group: ESBL-np strains have been selected among our clinical isolates as the control group. The repeated cultures were also excluded.

Clinical microbiology studies: Study and control group microorganisms have been isolated from clinical samples by routine microbiologic procedures. Isolated microorganisms were further examined with the BD Phoenix™ 100 Automated Microbiology System (Becton Dickson, USA) and Oxoid combination disk test methods. The inoculated Phoenix™ panels were placed into the Phoenix™ instrument for incubation and continuous reading. Aztreonam and extensive cephalosporin resistant members of Enterobacteriaceae were classified as "possibleESBL-p" strains, whereas sensitive members were classified as "ESBL-np".

Antibiotic susceptibility tests were performed by agar dilution test as described by Leber [13]. The following antimicrobial agents were used in the test: All beta-lactams including aztreonam cephalosporins, and carbapenems, amikacin-gentamicin-netilmicin (the aminoglycosides category), ciprofloxacin-levofloxacin-moxifloxacin (the fluoroquinolone category), ertapenem-meropenem (the carbapenem category), sulfamethoxazole/trimethoprim, piperacillin/tazobactam, colistin, nitrofurantoin, fosfomycin, and tygecycline.

ESBL-p strains detected by double disk synergy methods described by Kaur et al. [14].

Beta-lactam resistance was not evaluated in this study because, the aim of this study is to detect the correlations between ESBL positivity and other MDR. These resistances have only been used to detect and control ESBL positivity.

The relationship between ESBL positivity and the extensiveness and the co-existence patterns of resistance amongst MDR to ESBL-RA in Enterobacteriaceae strains have been compared to the control group. ESBL-MDR mosaics have been created for this purpose.

Statistical analysis: Results were statistically analyzed by "Difference between two proportions for independent samples", and the mean age of patients has been analyzed by t-test for independent samples.

Results

Demographic and clinical characteristics of patients' isolated ESBL-p and ESBL-np group microorganisms have been outlined in **Table 1**.

Table 1 Demographic and clinical characteristics of patients' isolated ESBL-Producer and non-producer control group microorganisms.

Characteristics		ESBL-p (n=170) (%)	ESBL-np (n=170) (%)	p Values
Age	Mean	56.22	48.72	<0.0001
	Max.	99	95	
	Min.	0.014	0	
	Std. Deviation (SD)	24.58	27.09	
	Std. Error of Mean (SEM)	1.89	2.08	
Gender	Male	63 (37.06)	48 (28.24)	0.3306
	Female	107 (62.94)	122 (71.76)	0.156
Microorganisms	<i>E coli</i>	122 (71.76)	125 (73.53)	0.7553
	Klebsiella	48 (28.24)	44 (25.88)	0.7998
Patient's source	Outpatient	56 (32.94)	97 (57.06)	0.0046
	Inpatient	114 (67.05)	73 (42.94)	0.0013
Samples	Pus	11 (6.47)	9 (5.29)	0.9128
	Aspiration	6 (3.53)	6 (3.53)	
	Sputum	13 (7.65)	3 (1.76)	0.7156
	Urine	123 (72.35)	130 (76.47)	0.4533
	Blood	10 (5.88)	12 (7.06)	0.9123
	Catheter tip	4 (2.35)	3 (1.76)	0.9591
	CSF	1	0	
	Urethral discharge	1 (0.59)	2 (1.18)	0.7908
	Vaginal samp.	0	5	
Origin	Intensive care	3	0	
	Hospital Ward	111 (65.29)	170 (100)	<0.0001

Individual resistance to ESBL-RA in ESBL-p and ESBL-np Enterobacteriaceae strains has been shown in **Table 2**.

Table 2 Individual resistance to ESBL-RA in ESBL-p and ESBL-np in Enterobacteriaceae strains.

	ESBL-p		ESBL-np		p
	R/S	%	R/S	%	
SXT	113/53	68.20%	51/119	30.00%	<0.0001
TZP	47/120	27.70%	08/161	4.73%	<0.0001
FQ	118/52	69.40%	40/130	23.50%	<0.0001
NIT	Jun-79	23.50%	2/103	1.90%	<0.0001
AG	58/109	34.70%	10/159	5.30%	<0.0001
CAR	32/137	18.90%	9/161	5.20%	<0.0001
FOF	Feb-63	3.00%	1/132	0.07%	0.2552
TGC	Apr-41	8.80%	NOT TESTED		
COL	6/164	3.50%	0/170	0.00%	

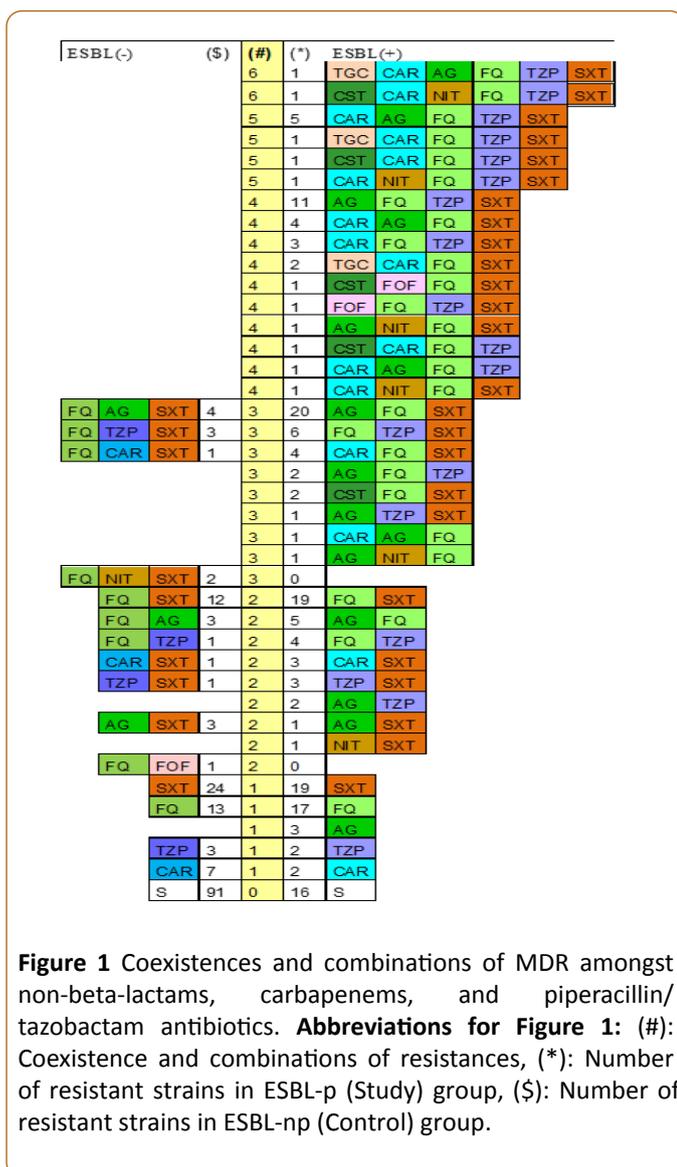
The extensiveness of MDR's are outlined in **Table 3**.

Table 3 Extensiveness of MDR pattern in ESBL-p and ESBL-np GNB strains.

Co-resistance patterns	ESB-p (%)	ESBL-np (%)	p Values
Six-drug MDR	2	0	
Five-drug MDR	8	0	
Four-drug MDR	26	0	
Three-drug MDR	37 (21.76)	10 (5.88)	0.2561
Two -drug MDR	38 (22.35)	22 (12.94)	0.3733
One-drug MDR	43 (25.29)	47 (27.65)	0.8006
No-drug MDR (All sensitive to non β -lactams)	16 (9.41)	91 (53.33)	0.0015

As shown in **Table 3**, there are no strains in the three-drug to six-drug resistances range in the ESBL-np group.

Co-existences of MDR and their combinations amongst ESBL-RA antibiotics have been outlined in **Figure 1**.



Discussion

The aims of this study are to define the phenotypic MDR patterns of ESBL-RA's in ESBL-p and ESBL-np Enterobacteriaceae strains and to show whether ESBL positivity is a dominant characteristic especially on resistance to non-beta-lactam antibiotics.

The ESBL-p and control groups were largely similar in demographic and clinical characteristic in the study, except for only two minor statistical differences. The mean age of the study group was older than the control group (56.22 vs 48.72). Additionally, there was a significant difference between the patient's sources, meaning that a higher percentage of ESBL-p

samples were collected from inpatient clinics. However, on the contrary, ESBL-np strains were mainly isolated from outpatient sources.

The higher individual resistance prevalence to all ESBL-RA have been detected in the ESBL-p group. All isolates of the ESBL-np group were sensitive to colistin. On the contrary; only six (3.5%) colistin-resistant strains have been isolated in ESBL-p group.

Our ESBL-p strains showed 8.8% resistance to tigecycline, but it was not possible to collect susceptibility test results to this antibiotic for the ESBL-np group because of the institutionally restricted antibiotic prescribing policies. The least resistant ESBL-RA's detected were *colistin*, nitrofurantoin, and fosfomycin in ESBL-np group, respectively. Fosfomycin was also the least resistant antibacterial agents in the ESBL-p group. These results reveal excellent treatment options still exist, particularly for lower urinary tract infections. Similar resistant patterns have been published previously [12-15].

A total of nine individual types of resistance in different combinations have been detected in our ESBL-p group.

A statistical difference between ESBL-p and ESBL-np in the extensiveness of MDR has been also detected. A total of 36 (21%) ESBL-p strains showed four-to-six drug MDR to ESBL-RA, while we could not detect any resistance strains with similar characteristics in the control group. When the was gradually decreased from three-drug to one-drug resistance, the proportional difference between the -p and -np groups also decreased so that one drug resistance in ESBL-np was higher than ESBL-p.

Ninety-one strains of our ESBL-NP were totally sensitive to ESBL-RA while only 16 strains were in the same characteristics in ESBL-p group (53.3% to 9.4% respectively: $p=0.0015$)

Our study group of enteric isolates had a total of 33 different resistance combination coexistences, compared with 11 different combinations in the control groups [16].

It was found that the most prevalent triplet combinations of MDR in ESBL-p strains was trimethoprim/sulfamethoxazole+fluoroquinolones+aminoglycosides in combination, and this was the ten times more frequent than in the control group (43 vs 4 strains). The second most prevalent triplet resistance was trimethoprim/sulfamethoxazole+fluoroquinolones+ piperacillin /tazobactam and this combination was also ten times more frequent than in the control group (31 vs 3 strains). Moreover, in the third most frequent combination was trimethoprim/sulfamethoxazole+fluoroquinolones and carbapenems (22 vs 1 strains).

A series of MDR-including carbapenem-resistant OXA-48 positive enterobacterial isolates were recovered from Turkey has been previously reported [17]. Most of the reported isolates were resistant to fluoroquinolones, trimethoprim/sulfamethoxazole, and aminoglycosides in addition to carbapenems and the other beta-lactams. These strains were sensitive to other antibiotics not indicated above. It is highly possible that the most of our strains also comprise OXA plasmids as a part of its epidemics.

Our three most prevalent resistant pattern-containing strains may carry different accessory resistance characteristics such as colistin, fosfomicin, nitrofurantoin and tigecycline resistance.

One of the most remarkable co-existence of resistance is carbapenemase containing ESBL-RA resistance combinations in our enterobacterial isolates. This reminds us that our ESBL-p strains should be classified into two different groups according to their carbapenemase producing/non-producing characteristics. One-half of our ESBL-p total isolates contains carbapenemase-producing combinations (81 isolates: 47.6%), whereas the other half are ESBL-p without carbapenemase production.

In this study, we presented only phenotypic resistance characteristics rather than genetic analysis. However, it is highly possible that the dominant characteristics of these carbapenemases controls and manages the ESBL-RA resistance. Qeenan and Bush denominated carbapenemases as “versatile beta-lactamases” because of their broad activity in a wide range of beta-lactams [18,19]. It is apparent that this versatility is not only limited to beta-lactams, but also to non-beta-lactams.

Both carbapenemases and other ESBL genes may harbor a set of non-beta-lactam resistance genes such as fluoroquinolones, aminoglycosides and/or trimethoprim/sulfamethoxazole resistances [7,20,21].

On the other hand, in this study, we presented the first MDR results in Enterobacteriaceae strains for Cyprus. Country-wide susceptibility test results are required to understand the precise resistance epidemiology.

Investigations of the genotypic characteristics of our strains will a topic of further studies.

Conclusion

This study showed us that ESBL positivity controls and commands MDR to ESBL-RA in enterobacterial isolates. This control is stronger in longer co-resistance, while on the contrary, the shorter co-resistance is independent of ESBL positivity. When the ESBL-p strains are classified as carbapenemase and non-carbapenemase producing in origin, there is no significant coexistence difference between these subgroups. In daily medical practices, time-consuming laboratory test methods can be beneficial for choosing successful treatment options. Immediate ESBL test results are also necessary, and these results may not only direct us to avoid beta-lactams but also other options such as trimethoprim/sulfamethoxazole, fluoroquinolones, and aminoglycosides.

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Transparency Declaration

We affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

References

1. Pitout JD, Laupland KB (2008) Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis* 8: 159-166.
2. Pitout JD (2010) Infections with extended-spectrum beta-lactamase-producing Enterobacteriaceae: changing epidemiology and drug treatment choices. *Drugs* 70: 313-33.
3. Oteo J, Pérez-Vázquez M, Campos J (2010) Extended-spectrum [beta]-lactamase producing *Escherichia coli*: changing epidemiology and clinical impact. *Curr Opin Infect Dis* 23: 320-326.
4. Magiorakos AP, Srinivasan A, Carey RB, Carmel Y, Falagas ME, et al. (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 18: 268-81.
5. Van Hoek AH, Mevius D, Guerra B, Mullany P, Robberts AP (2011) Acquired antibiotic resistance genes: an overview. *Front Microbiol* 28: 203.

6. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL (2012) Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: an evolving crisis of global dimensions. *Clin Microbiol Rev* 25: 682-707.
7. Carrër A, Poirel L, Yilmaz M, Akan OA, Feriha C, et al. (2010) Spread of OXA-48-encoding plasmid in Turkey and beyond. *Antimicrob Agents Chemother* 54: 1369-1373.
8. Sun J, Deng Z, Yan A (2014) Bacterial multidrug efflux pumps: mechanisms, physiology and pharmacological exploitations. *Biochem Biophys Res Commun* 453: 254-67.
9. Bradford PA (2001) Extended-spectrum beta-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 14: 933-951.
10. Paterson DL, Mulazimoglu L, Casellas JM, Ko W, Goossens H, et al. (2000) Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. *Clin Infect Dis* 30: 473-478.
11. Woodford N, Kaufmann ME, Karisik E, Hartley JW (2006) Molecular epidemiology of multiresistant *Escherichia coli* isolates from community-onset urinary tract infections in Cornwall, England. *J Antimicrob Chemother* 59: 106-109.
12. Sohn KM, Kang CI, Joo EJ, Ha YE, Chung DR, et al. (2011) Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in *Proteus mirabilis* bacteremia. *Korean J Intern Med* 26: 89-93.
13. Leber AL (2016) Agar dilution MIC test. In *Clinical Microbiology Procedures Handbook, Fourth Edition*. ASM Press, Washington.
14. Kaur J, Chopra S, Sheevani, Mahajan G (2013) Modified Double Disc Synergy Test to Detect ESBL Production in Urinary Isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *Journal of Clinical and Diagnostic Research* 7: 229-233.
15. Paltansing S, Kraakman ME, Ras JM, Wessels E, Bernards AT (2013) Characterization of fluoroquinolone and cephalosporin resistance mechanisms in Enterobacteriaceae isolated in a Dutch teaching hospital reveals the presence of an *Escherichia coli* ST131 clone with a specific mutation in *parE*. *J Antimicrob Chemother* 68: 40-45.
16. Crémet L, Caroff N, Dauvergne S, Reynaud A, Lepelletier D, et al. (2011) Prevalence of plasmid-mediated quinolone resistance determinants in ESBL Enterobacteriaceae clinical isolates over a 1-year period in a French hospital. *Pathol Biol* 59: 151-156.
17. Briales A, Rodríguez-Martínez JM, Velasco C, Reynaud A, Lepelletier D, et al. (2012) Prevalence of plasmid-mediated quinolone resistance determinants *qnr* and *aac(6')-Ib-cr* in *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases in Spain. *Int J Antimicrob Agents* 39: 431-434.
18. Dandachi I, Salem Sokhn E, Najem E, Azar E, Daoud Z, et al. (2016) Carriage of beta-lactamase-producing Enterobacteriaceae among nursing home residents in North Lebanon. *Int J Infect Dis* 45: 24-31.
19. Queenan AM, Bush K (2007) Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev* 20: 440-458.
20. Machado E, Coque TM, Cantón R, Baquero F, Sousa JC, et al. (2006) Portuguese Resistance Study Group. Dissemination in Portugal of CTX-M-15-, OXA-1-, and TEM-1-producing Enterobacteriaceae strains containing the *aac(6')-Ib-cr* gene, which encodes an aminoglycoside- and fluoroquinolone-modifying enzyme. *Antimicrob Agents Chemother* 50: 3220-3221.
21. Girlich D, Bouihat N, Poirel L, Benouda A, Nordmann P (2014) High rate of faecal carriage of extended-spectrum beta-lactamase and OXA-48 carbapenemase-producing Enterobacteriaceae at a university hospital in Morocco. *Clin Microbiol Infect* 20: 350-354.