Scientific Research Publishing

Advances in Microbiology, 2024, 14, 115-121
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Phenotypic Detection of Enterobacterales Strains Susceptible of Producing OXA-48 Carbapenemase

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How to cite this paper: Seck, A., Diop, A., Ndiaye, B., Dieng, A., Ba, A., Diop, A., Douala-Djemba, C.M. and Diallo, T.A. (2024) Phenotypic Detection of Enterobacterales Strains Susceptible of Producing OXA-48 Carbapenemase. *Advances in Microbiology*, **14**, 115-121.
https://doi.org/10.4236/aim.2024.142009

Received: October 27, 2023
Accepted: February 16, 2024
Published: February 19, 2024

Abstract

Background: Nowadays, emergence of Carbapenemase-Producing Enterobacterales (CPE) throughout the world has become a public health problem, especially in countries with limited resources. In recent years, CPE of type OXA-48 (Ambler class D) have been identified in Dakar. The aim of this study was to evaluate the phenotypic detection of OXA-48 CPE using a temocillin disc (30 µg). **Methodology:** A retrospective study was carried out at Medical Biology Laboratory of Pasteur Institute in Dakar on Ertapenem-Resistant Enterobacterales (ERE) strains isolated from 2015 to 2017. These strains were then tested with a 30 µg temocillin disc. Any strain resistant to temocillin (inhibition diameter < 12 mm) was considered to be OXA-48 CPE. Confirmation of OXA-48 CPE was performed by PCR and sequencing. **Results:** Forty-one ERE isolated during the study period were tested, of which 34 (82.9%) were OXA-48 based on phenotypic detection using temocillin disc and confirmed by PCR (100%). OXA-48 CPE strains detected were composed of *Klebsiella pneumoniae* (n = 14; 41.2%), *Enterobacter cloacae* (n = 8; 23.5%), *Escherichia coli* (n = 7, 20.5%), *Citrobacter freundii* (n = 3; 8.8%), *Cronobacter sakazakii* (n = 1; 3%) and *Morganella morganii* (n = 1; 3%). **Conclusion:** Temocillin resistance has a good positive predictive value for detecting OXA-48 CPE by phenotypic method, confirmed by PCR. Temocillin is therefore a good marker for detection of OXA-48 CPE except *Hafnia alvei*.

Keywords

Ertapenem, Temocillin, Phenotypic Detection, Carbapenemase-Producing Enterobacterales, OXA-48

1. Introduction

Bacterial resistance to antibiotics is steadily increasing and constitutes a global public health problem. According to estimates by World Health Organization (WHO), human and economic impacts of antimicrobial resistance between now and 2050 will be considerable [1]. Over last ten years, emergence and spread of Carbapenem-Resistant Enterobacterales (CRE) and, in particular, *Klebsiella pneumoniae*, have become worldwide [2].

Carbapenems are antibiotics of choice for serious infections caused by multi-resistant gram-negative bacteria [3]. CRE can cause treatment failures and constitute a major public health problem [4]. Resistance due to production of a carbapenemase is transferred horizontally among Enterobacteriaceae, and there are many types of carbapenemases: clavulanic acid-inhibited ß-lactamases (Class A: KPC, NMC, IMI, SME and GES), metallo-betalactamases (Class B: IMP, VIM, NDM, GIM, SPM and SIM) and extended-spectrum oxacillinases (Class D: OXA-48-like) [5]. With exception of OXA-48, these enzymes confer high resistance to most beta-lactam compounds such as penicillins and cephalosporins, but affect carbapenems to a variable extent [6]. OXA-type carbapenemases are considered to be penicillinases capable of hydrolyzing oxacillin and cloxacillin and are weakly inhibited by clavulanic acid and EDTA. OXA-48 is one of the few members of this family to have significant carbapenem hydrolysis activity [7].

OXA-48 carbapenemases have been frequently reported in North African and other Mediterranean countries [6] [7]. In Morocco, an increased spread of OXA-48-producing Enterobacteriaceae isolates linked to healthcare-associated infections has been reported [8] [9].

In Senegal, emergence of plasmid-encoded $bla_{\text{OXA-48}}$ gene has been reported in multi-resistant Enterobacteriaceae of community and hospital origin in Dakar [10]. Detection of OXA-48 carbapenemases remains difficult due to multitude of phenotypes and resistance mechanisms [11]. A phenotypic test for detection of OXA-48 CPE has been proposed with temocillin disc. Temocillin is a derivative of ticarcillin (6-α-methoxy ticarcillin) which has a narrow spectrum for enterobacteria and is not hydrolyzed by β-lactamases (ESBL, AmpC) and cephalosporinases. *Hafnia alvei* has natural resistance to temocillin [12].

The aim of this study was to use a phenotypic identification method to detect OXA-48 CPE in strains of Enterobacteriaceae isolated in Dakar.

2. Material and Methods

2.1. Bacterial Strains

This retrospective study was carried out at Medical Biology Laboratory of Pasteur Institute in Dakar on enterobacteria strains isolated from 2015 to 2017. Strains resistant to ertapenem (10 µg) with an inhibition diameter of less than 25 mm according to EUCAST 2020 recommendations were included in the study. Phenotypic detection of OXA-48 CPE was performed using temocillin

disc (30 µg).

2.2. Phenotypic Detection of Resistance to Temocillin

Antibiotic susceptibility profiling was performed using Mueller-Hinton agar diffusion method (Becton Dickinson, Heidelberg, Germany) with antibiotic disc temocillin (30 µg) according to guidelines of the European Committee of Antimicrobial Susceptibility Testing (EUCAST, 2020).

2.3. Molecular Confirmation

Presence of OXA-48 CPE was confirmed by PCR using following specific primers.

O48-F CCA AGC ATT TTT ACC CGC ATC KAC C

O48-R GYT TGA CCA TAC GCT GRC TGC G

PCR and sequencing were performed searching of $bla_{\text{OXA-48}}$ gene as described previously [13].

Control strain NCTC-13442 was used as a reference.

2.4. Data Analysis

Data were entered and analysed using Excel 2018 and logiciel R. We estimated the sensitivity, specificity and kappa coefficient of the phenotypic test in reference to PCR.

3. Results

3.1. Bacterial Strains

Forty-one strains of enterobacteria isolated from various pathological samples were analysed for this study. These strains were mainly composed of *K. pneumoniae* (n = 16), *E. coli* (n = 11) and *E. cloacae* (n = 9) (**Table 1**).

Table 1. Distribution of enterobacteria strains tested.

Strains	Number	%
K. pneumoniae	16	39
E. coli	11	26.9
E. cloacae	9	22
C. freundii	3	7.3
C. sakazakii	1	2.4
M. morganii	1	2.4
Total	41	100

3.2. Resistance to Ertapenem

Antibiotic susceptibility profiling by diffusion method showed that all strains of enterobacteria were resistant to ertapenem. Inhibition diameters range from 8 to 21 mm depending on the species (**Table 2**).

Table 2. Enterobacteria strains resistant to ertapenem.

Strains	Profil to ertapenem	
	Diameter range (mm)	Resistant
K. pneumoniae	8 - 20	16
E. coli	8 - 23	11
E. cloacae	8 - 17	9
C. freundii	10 - 15	3
M. morganii	8	1
C. sakazakii	10	1

3.3. Resistance to Temocillin

Of the 41 strains resistant to ertapenem, 34 isolates were resistant to temocillin (Table 1), and therefore suspected of being EPC probably type OXA-48.

These isolates included *K. pneumoniae* (n = 14), *E. cloacae* (n = 8), *E. coli* (n = 7), *C. freundii* (n = 3), *C. sakazakii* (n = 1) and *M. morganii* (n = 1).

3.4. Resistance of PCR and Sequencing of $bla_{\text{OXA-48}}$

All strains both resistant to ertapenem and temocillin harbored OXA-48 gene (Table 2). Sequencing confirmed presence of OXA-48 gene in all 34 temocillin-resistant isolates (Table 3): GenBank accession numbers NG049762.1 (n = 31) and NG 049482.1 (n = 3).

Table 3. Temocillin-resistant strains of enterobacteria harboring the OXA-48 gene.

Strains	Profil to temocillin				OXA-48 gene	
	Diameter range (mm)	R	I	S	Positive	Negative
K. pneumoniae	6 - 26	14	0	2	14	2
E. coli	6 - 26	7	1	3	7	4
E. cloacae	6 - 24	8	0	1	8	1
C. freundii	6	3	0	0	3	0
M. morganii	6	1	0	0	1	0
C. sakazakii	6	1	0	0	1	0
Total		34	1	6	34	7

R: resistant, I: intermediate, S: sensitive.

3.5. Comparison Temocillin Resistance versus PCR

This study showed a sensitivity of 100% (95% CI: 0.90 - 1.0), a specificity of 100% (95% CI: 0.54 - 1.0), and a kappa coefficient equal to 1 for the phenotypic test compared with PCR (Table 4).

Table 4. Comparison of phenotypic detection with temocillin disk versus.

	PCR (reference test)		Total
	OXA-48 presence	OXA-48 absence	
Temocillin R	34	0	34
Temocillin S or I	0	7	7
Total	34	7	41

4. Discussion

The aim of this study was to evaluate phenotypic detection of OXA-48 CPE strains isolated from 2015 to 2017 with a temocillin disc (30 µg). Confirmation was made by PCR for reschearch of OXA-48 gene.

Forty-one enterobacteria strains resistant to ertapenem (inhibition diameter < 22 mm, EUCAST 2020) were analyzed. Temocillin resistance was found in 83% of isolates (n = 34), suggesting a probable CPE type OXA-48. Molecular analysis of these strains confirmed the presence of OXA-48 gene in all temocillin-resistant isolates. For strains resistant to ertapenem, other carbapenem resistance genes (KPC, IMP, VIM, NDM, IMI, GES, etc.) should also be checked.

In Senegal, the circulation of enterobacteria strains harboring $bla_{\text{OXA-48}}$ gene isolated in community and hospital settings has been reported since 2011 [10]. These CPE type OXA-48 are endemic in several African countries, including North Africa, Türkiye, Arabian Peninsula and India [14].

In France in 2020, OXA-48 carbapenemases accounted for 63.3% of EPCs in circulation according to data from National Reference Center [15].

In this study, 14 strains of *K. pneumoniae* (41%) produced carbapenemase type OXA-48. The spread of *K. pneumoniae* strains harboring genes encoding resistance to carbapenems has been described throughout the world [16].

The increasing use of carbapenems in clinical practice is being followed by emergence of CPE, which are increasingly being detected throughout the world, and this trend has been observed in Senegal. Emergence of carbapenemases acquired from enterobacteria appeared early in Senegal (2008) after introduction of imipenem, and their frequency is probably underestimated, partly because of limited number of bacteriology laboratories equipped with qualified staff [17]. Multiple antibiotic resistance is prevalent among enterobacteria strains isolated in rural communities [18], but especially in hospitals in Dakar where emergence of CPE harboring the $bla_{\text{OXA-48}}$ gene is a major concern.

Sensitivity and specificity of phenotypic test using temocillin disk were 100% in reference to PCR detection of the $bla_{\text{OXA-48}}$ gene. The good sensitivity and specificity of phenotypic test with temocillin may be a means of rapidly detecting ertapenem-resistant enterobacteria strains likely to be OXA-48 carbapenemase producers, and of managing patients infected with these strains. In fact, this test does not require any investment on the part of the laboratory, compared to the molecular biology technique, which is not accessible to most laboratories in de-

veloping countries, and also to rapid diagnostic test, which despite its lower cost is not yet within the reach of these laboratories. All strains of enterobacteria resistant to ertapenem and temocillin require confirmation of OXA-48 carbapenemase production by rapid diagnostic method (immunoassay) or molecular technique.

This study has some limitations, notably the fact that it only detects OXA-48 type carbapenemases and therefore cannot identify other classes of carbapenemases.

However, some bacterial species are naturally resistant to temocillin (e.g. *Hafnia alvei*), and other (fairly rare) mechanisms can induce resistance to this antibiotic.

5. Conclusion

The phenotypic test with temocillin disk has shown good sensitivity and specificity for detecting ertapenem-resistant enterobacteria strains likely to produce an OXA-48 carbapenemase. However, identification of carbapenemase type must be carried out by a rapid diagnostic method (immunoassay) or by PCR.

Authors' Contributions

AS designed the study and revised manuscript version. AD and BN participated susceptibility testing and molecular test. CT carried out statistical analyses. All authors have read and approved final version of manuscript.

Acknowledgements

Medical Biology Laboratory of Pasteur Institute of Dakar (IPD).

Conflicts of Interest

The authors declare no conflict of interest.

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