

Comparison of stool samples and rectal swabs with and without pre-enrichment for the detection of third-generation cephalosporin-resistant *Enterobacteriales* (3GCREB)

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Online Resource 1 Drug susceptibilities of 3GCREB isolates recovered by the four approaches and compared to each other

Strain characteristics	3GCREB isolates recovered by														
	Overall (n=97)			Stool without pre-enrichment (A) (n=68)			Stool with pre-enrichment (B) (n=81)			Rectal swab without pre-enrichment (C) (n=63)			Rectal swab with pre-enrichment (D) (n=75)		
Drug Susceptibility ^a	MIC ₅₀ ^a	MIC ₉₀ ^a	I/R ^a (n, %)	MIC ₅₀ ^a	MIC ₉₀ ^a	I/R ^a (n, %)	MIC ₅₀ ^a	MIC ₉₀ ^a	I/R ^a (n, %)	MIC ₅₀ ^a	MIC ₉₀ ^a	I/R ^a (n, %)	MIC ₅₀ ^a	MIC ₉₀ ^a	I/R ^a (n, %)
Piperacillin/Tazobactam	64	>128	60 (61.9%)	16	>128	36 (52.9%)	32	>128	46 (56.8%)	16	>128	33 (52.4%)	64	>128	46 (61.3%)
Cefpodoxime	>8	>8	97 (100%)	>8	>8	68 (100%)	>8	>8	81 (100%)	>8	>8	63 (100%)	>8	>8	75 (100%)
Cefotaxime	>64	>64	92 (94.8%)	>64	>64	66 (97.1%)	>64	>64	77 (95.1%)	>64	>64	62 (98.4%)	>64	>64	73 (97.3%)
Ceftazidime	16	>64	76 (78.4%)	16	>64	53 (77.9%)	16	>64	65 (80.2%)	16	>64	49 (77.8%)	16	>64	59 (78.7%)
Ertapenem	<0.5	2	15 (15.5%)	<0.5	1	9 (13.2%)	<0.5	2	14 (17.3%)	<0.5	1	10 (15.9%)	<0.5	1	11 (14.7%)
Imipenem	<0.25	1	4 (4.1%)	<0.25	1	3 (4.4%)	<0.25	1	4 (4.9%)	<0.25	0.5	3 (4.8%)	<0.25	1	3 (4.0%)
Meropenem	<0.25	<0.25	2 (2.1%)	<0.25	<0.25	1 (1.5%)	<0.25	<0.25	2 (2.5%)	<0.25	<0.25	1 (1.6%)	<0.25	<0.25	1 (1.3%)
Ciprofloxacin	<0.25	>4	38 (39.2%)	<0.25	>4	30 (44.1%)	<0.25	>4	33 (40.7%)	<0.25	>4	25 (39.7%)	<0.25	>4	29 (38.7%)
Trimethoprim/Sulfamethoxazole	<20	>320	43 (44.3%)	>320	>320	35 (51.5%)	<20	>320	38 (46.9%)	<20	>320	30 (47.6%)	<20	>320	34 (45.3%)

^a MIC₅₀/MIC₉₀ estimates the antibiotic concentration (mg/liter) that inhibits 50% (MIC₅₀) and 90% (MIC₉₀) of tested bacterial isolates; I/R, number of isolates considered to be intermediate (I) or resistant (R) to the indicated antimicrobial agent according to EUCAST clinical MIC breakpoints.

* p-value was obtained using χ^2 test and accounts for the comparison of 3GCREB isolates recovered by the four approaches.

3GCREB, third-generation cephalosporin-resistant *Enterobacteriales*

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Online Resource 2 Strain characteristics of ESBL-E isolates recovered by the four approaches and compared to each other

Strain characteristics	Overall (n=54)	ESBL-E isolates recovered by													
		Stool without pre-enrichment (A) (n=47)			Stool with pre-enrichment (B) (n=49)			Rectal swab without pre-enrichment (C) (n=43)			Rectal swab with pre-enrichment (D) (n=44)				
Species															
<i>Escherichia coli</i>	45 (83.3%)	42 (89.4%)			41 (83.7%)			37 (86.0%)			37 (84.1%)				
<i>Klebsiella pneumoniae</i>	3 (5.6%)	3 (6.4%)			3 (6.1%)			2 (4.7%)			2 (4.5%)				
<i>Klebsiella aerogenes</i>	2 (3.7%)	0			2 (4.1%)			1 (2.3%)			1 (2.3%)				
<i>Enterobacter cloacae</i>	3 (5.6%)	2 (4.3%)			3 (6.1%)			3 (7.0%)			3 (6.8%)				
<i>Citrobacter farmeri</i>	1 (1.9%)	0			0			0			1 (2.3%)				
ESBL genes															
CTX-M-1 (no. (% of ESBL))	33 (61.1%)	30 (63.8%)			31 (63.3%)			29 (67.4%)			28 (63.6%)				
CTX-M-9 (no. (% of ESBL))	9 (16.7%)	7 (14.9%)			8 (16.3%)			6 (14.0%)			8 (18.2%)				
Unknown (no. (% of ESBL))	12 (22.2%)	10 (21.3%)			10 (20.4%)			8 (18.6%)			8 (18.2%)				
Drug Susceptibilities^a															
	MIC50	MIC90	I/R (n, %)	MIC50	MIC90	I/R (n, %)	MIC50	MIC90	I/R (n, %)	MIC50	MIC90	I/R (n, %)	MIC50	MIC90	I/R (n, %)
Piperacillin/Tazobactam	8	>128	19 (35.2%)	8	>128	16 (34.0%)	8	>128	16 (32.7%)	8	>128	14 (32.6%)	8	>128	16 (36.4%)
Cefpodoxime	>8	>8	54 (100%)	>8	>8	47 (100%)	>8	>8	49 (100%)	>8	>8	43 (100%)	>8	>8	44 (100%)
Cefotaxime	>64	>64	53 (98.1%)	>64	>64	46 (97.9%)	>64	>64	48 (98.0%)	>64	>64	42 (97.7%)	>64	>64	44 (100%)
Ceftazidime	8	>64	37 (68.5%)	8	>64	32 (68.1%)	8	>64	35 (71.4%)	8	>64	30 (69.8%)	8	>64	30 (68.2%)
Ertapenem	<0.5	<0.5	2 (3.7%)	<0.5	<0.5	2 (4.3%)	<0.5	<0.5	2 (4.1%)	<0.5	<0.5	2 (4.7%)	<0.5	<0.5	2 (4.5%)
Imipenem	<0.25	<0.25	0	<0.25	<0.25	0	<0.25	<0.25	0	<0.25	0.5	0	<0.25	0.5	0
Meropenem	<0.25	<0.25	0	<0.25	<0.25	0	<0.25	<0.25	0	<0.25	<0.25	0	<0.25	<0.25	0
Ciprofloxacin	>4	>4	32 (59.3%)	>4	>4	28 (59.6%)	>4	>4	28 (57.1%)	2	>4	23 (53.5%)	>4	>4	26 (59.1%)
Trimethoprim/Sulfamethoxazole	>320	>320	37 (68.5%)	>320	>320	33 (70.2%)	>320	>320	33 (67.3%)	>320	>320	29 (67.4%)	>320	>320	32 (72.7%)

^a MIC₅₀/MIC₉₀ estimates the antibiotic concentration (mg/l) that inhibits 50% (MIC₅₀) and 90% (MIC₉₀) of tested bacterial isolates; I/R, number of isolates considered to be intermediate (I) or resistant (R) to the indicated antimicrobial agent according to EUCAST clinical MIC breakpoints.

ESBL-E, extended-spectrum-beta-lactamase-producing *Enterobacteriales*

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Online Resource 3 Distribution of identified species and resistance mechanisms among the 97 3GCREB study isolates

	ESBL (n (%))				Other resistance mechanism (n(%))					Total (n (%)) ⁴
	CTX-M-1 group ²	CTX-M-9 group ²	unknwon ESBL ²	Total ESBL ¹	AmpC ¹	Hyper K1 ¹	bla _{IMP} ¹	bla _{VIM} ¹	SHV ¹	
<i>Escherichia coli</i>	29 (64.4)	7 (15.6)	9 (20.0)	45 (100)	-	-	-	-	-	45 (46.4)
<i>Klebsiella pneumoniae</i>	2 (66.7)	-	1 (33.3)	3 (50.0)	1 (16.7)	-	-	-	2 (33.3)	6 (6.2)
<i>Klebsiella oxytoca</i>	-	-	-	-	-	1 (100)	-	-	-	1 (1.0)
<i>Klebsiella aerogenes</i>	1 (50.0)	-	1 (50.0)	2 (50.0)	2 (50.0)	-	-	-	-	4 (4.1)
<i>Enterobacter cloacae</i>	1 (33.3)	2 (66.7)	-	3 (37.5)	5 (62.5)	-	-	-	-	8 (8.2)
<i>Other Enterobacter spp.</i>	-	-	-	-	4 (100)	-	-	-	-	4 (4.1)
<i>Citrobacter freundii</i>	-	-	-	-	17 (89.5)	-	-	2 (10.5)	-	19 (19.6)
<i>Citrobacter braakii</i>	-	-	-	-	4 (100)	-	-	-	-	4 (4.1)
<i>Other Citrobacter spp.</i>	-	-	1 (100)	1 (50.0)	1 (50.0)	-	-	-	-	2 (2.1)
<i>Hafnia alvei</i>	-	-	-	-	2 (100)	-	-	-	-	2 (2.1)
<i>Serratia marcescens</i>	-	-	-	-	-	-	2 (100)	-	-	2 (2.1)
Total (n (%))	33 (61.1)³	9 (16.7)³	12 (22.2)³	54 (55.7)⁴	36 (37.1)⁴	1 (1.0)⁴	2 (2.1)⁴	2 (2.1)⁴	2 (2.1)⁴	97 (100)

¹Percentage based on all isolates of the named species

²Percentage based on total number of ESBL positive isolates of the named species

³Percentage based on total number of ESBL positive isolates

⁴Percentage based on all 97 isolated 3GCREB

3GCREB, third-generation cephalosporin-resistant *Enterobacteriales*; ESBL-E, extended-spectrum-beta-lactamase-producing *Enterobacteriales*

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Online Resource 4 Patient characteristics of 3GCREB carriers detected by the four approaches and compared to each other

Patients characteristics ^a	Overall carrier (n=68)	3GCREB carrier recovered by			
		Stool without pre-enrichment (A) (n=44)	Stool with pre-enrichment (B) (n=56)	Rectal swab without pre-enrichment (C) (n=42)	Rectal swab with pre-enrichment (D) (n=54)
Age (years)	58.9 ± 22.2	59.1 ± 22.8	59.8 ± 21.5	56.1 ± 24.4	56.8 ± 23.4
No. Male (%)	40 (58.8%)	28 (63.6%)	33 (58.9%)	26 (61.9%)	31 (57.4%)
Clinical setting (no. (%))					
Internal Medicine	33 (48.5%)	21 (47.7%)	27 (48.2%)	20 (47.6%)	28 (51.9%)
Surgery	8 (11.8%)	4 (9.1%)	7 (12.5%)	3 (7.1%)	5 (9.3%)
Intensive Care Unit	13 (19.1%)	10 (22.7%)	12 (21.4%)	8 (19.0%)	10 (18.5%)
Pediatrics	5 (7.4%)	3 (6.8%)	3 (5.4%)	4 (9.5%)	5 (9.3%)
Others	9 (13.2%)	6 (13.6%)	7 (12.5%)	7 (16.7%)	6 (11.1%)

^a Calculation is based on a patients level (i.e., patients with more than one positive sample were only included in the calculation once).

* p-value by χ^2 test was calculated for 3GCREB carriers detected by the four approaches

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Online Resource 5 Patient characteristics of ESBL-E carriers detected by the four approaches and compared to each other

Patients characteristics ^a	Overall carrier (n=34)	ESBL-E carrier recovered by			
		Stool without pre-enrichment (A) (n=29)	Stool with pre-enrichment (B) (n=31)	Rectal swab without pre-enrichment (C) (n=27)	Rectal swab with pre-enrichment (D) (n=30)
Age (years)	59.2 ± 20.1	58.9 ± 17.4	58.1 ± 20.7	54.8 ± 20.1	56.6 ± 19.9
No. Male (%)	20 (58.8%)	20 (69.0%)	19 (61.3%)	18 (66.7%)	18 (60.0%)
Clinical setting (no. (%))					
Internal Medicine	21 (61.8%)	18 (62.1%)	19 (61.3%)	18 (66.7%)	20 (66.7%)
Surgery	3 (8.8%)	2 (6.9%)	3 (9.7%)	1 (3.7%)	1 (3.3%)
Intensive Care Unit	6 (17.6%)	6 (20.7%)	5 (16.1%)	4 (14.8%)	5 (16.7%)
Pediatrics	1 (2.9%)	0	1 (3.2%)	1 (3.7%)	1 (3.3%)
Others	3 (8.8%)	3 (10.3%)	3 (9.7%)	3 (11.1%)	3 (10.0%)

^a Calculation is based on a patients level (i.e., patients with more than one positive sample were only included in the calculation once).

* p-value by χ^2 test was calculated for ESBL-E carriers detected by the four approaches

ESBL-E, extended-spectrum-beta-lactamase-producing *Enterobacteriales*