Molecular Epidemiology of Carbapenem, Colistin and Tigecycline Resistant Enterobacteriaceae in Durban, South Africa

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Introduction and Purpose: The epidemiology and resistance mechanisms of carbapenem, tigecycline and colistin resistant Enterobacteriaceae isolated from the private health sector in Durban, South Africa (SA) were analyzed by whole genome sequencing (WGS).

Methods: Forty-seven Enterobacteriaceae clinical isolates with reduced susceptibility to carbapenems were collected between October 2012 and August 2013 from in-patients at ten hospitals in Durban. Micro-broth dilution, Modified Hodge's test (MHT), disc synergy, Vitek II and Carba NP tests were used to identify carbapenem resistant and carbapenemase producing strains. Real-time multiplex PCR was used to determine the presence of bla_{OXA-48}-like, bla_{KPC}, bla_{GIM}, bla_{SIM}, bla_{SPM}, bla_{VIM}, $blaI_{MP}$ and bla_{NDM-1}. The isolates were subjected to WGS (Illumina Miseq) with 300bp libraries prepared from genomic DNA. The raw reads were assembled with MIRA and SPADES and annotated with ResFinder (Center for Genomic Epidemiology) [2], ARG-ANNOT [3], RAST [4] and PGAP to identify all the antibiotic resistance genes in the isolates (Bioproject number PRJNA287968). The MLST of the isolates were determined with MLST 1.8 server (https://cge.cbs.dtu.dk/services/MLST/). RAST, ISFinder (http://-is.biotoul.fr/), BRIG [5] and BLASTn were used to determine the genetic environment of the carbapenemase genes. BLASTn multiple alignment was used to identify mutations in tigecycline and colistin resistance determining genes by aligning the sequences of the genes from resistant and susceptible isolates.

Results: The isolates comprised of *K. pneumoniae* (n=21), *S. marcescens* (n=12), *E. cloacae* (n=11), *Citrobacter freundii* (n=2), *Escherichia coli* (n=1) and *Klebsiella oxytoca* (n=1). *K. pneumoniae* sequence types were predominantly ST101 (n=14), followed by ST2017 (n=3) and single ST14, ST1478, ST2016 and ST323 strains whilst 10 of the *S. marcescens* strains were of the same clone (SA1) and from the same hospital ward. The *E. cloacae* strains were multi-clonal. Bla_{GES-5} was found only in *K. pneumoniae* ST101, but bla_{NDM-1} was identified in almost all the sequence types of *K. pneumoniae, E. cloacae, S. marcescens, C. freundii, E. coli* and *K. oxytoca*. Bla_{GES-5} was found mainly on class 1 integrons associated with pCHE-A like-plasmids (Fig.1A) while bla_{NDM-1} was borne on Tn*3*-like transposons linked to pNDM-HK-like plasmids (Fig.1B). Mutations in genes mediating resistance to colistin, *mgrB, phoP, phoQ, pmrAB* and *pmrHFIJKLM* as well as in genes mediating resistance to tigecycline, *acrABCR-TolC, marABCR, soxS/R, rob, ramAR* and *rarA*, were observed. Most isolates were pandrug resistant (Table 1)

Table 1: Minimum inhibitory concentrations of selected β-lactam and non-β-lactam antibiotics against the individual Enterobacteriaceae isolates

Isolate, ST	Minimum inhibitory concentrations (MIC), mg/L[1]															
	AMP	AMC	CXM	FOX[2]	CAZ	CTX	CPM	ETP	MEM	AMI	GEN	CIP	NFT	SXT	TGC	CST
K. pneumoniae																
D(UNN_S4), ST101	≥32	≥32	≥64	≥64	≥64	≥64	16	4	≥16	≥64	≥16	1	128	≤20	≥8	≥16
18_S10, ST101	≥32	≥32	≥64	≥64	≥64	≥64	≥64	≥8	4	≥64	≥16	4	128	≤20	≥8	≥16
29_S13, ST2017	≥32	≥32	≥64	≥64	≥64	≥64	16	4	4	≥64	≥16	≥4	32	≥320	≥8	≥16
35_S17, ST101	≥32	≥32	≥64	≥64	≥64	≥64	≥64	≥8	4	≥64	≥16	≥4	64	≥320	≥8	≥16
S. marcescens																
L (UNN_S12), SA1	≥32	≥32	≥64	≥64	≥64	≥64	≥64	≥8	≥16	≥64	≥16	≥16	128	≤20	≥8	≥16
K (UNN47_S11), SA1	≥32	≥32	≥64	≥64	≥64	≥64	≥64	≥8	≥16	≥64	≥16	≥4	128	≥320	≥8	≥16
45_S21, SA1	≥32	≥32	≥64	≥64	≥64	≥64	16	4	≥16	≥64	≥16	2	128	≥320	≥8	≥16
68_S34, SA1	≥32	≥32	≥64	≥64	≥64	≥64	≥64	≥8	≥16	≥64	≥16	≥4	128	≥320	≥8	≥16
E. cloacae																
F (UNN42_S6), ST121	≥32	≥32	≥64	≥64	≥64	≥64	16	≥8	≥16	≥64	≥16	2	128	≤20	≥8	≥16
65_S32, ST436	≥32	≥32	≥64	≥64	≥64	≥64	16	4	≥16	≥64	≥16	2	128	≤20	≥8	≥16
E. coli																
10_S4, ST167	≥32	≥32	≥64	≥64	≥64	≥64	16	4	≥16	≥64	≥16	1	64	≤20	≥8	≥16
C. freundii																
48_S23, ST63	≥32	≥32	≥64	≥64	≥64	≥64	16	≥8	≥16	≥64	≥16	≥4	32	≥320	≥8	≤0.5
K. oxytoca																
69_S35, ST170	≥32	≥32	≥64	≥64	≥64	≥64	16	4	≥16	≥64	≥16	1	128	≤20	2	≥16

[1] EUCAST resistant breakpoints are used throughout except for cefoxitin. MICs above this value indicate that the bacterial strain is resistant to the antibiotic: AMP=Ampicillin (R >8mg/L); AMC=Amoxicillin-clavulanic acid (R>8mg/L); CXM=Cefuroxime (R>8mg/L); FOX=Cefoxitin (R≥32); CAZ=Ceftazidime (R>4mg/L); CTX=Cefotaxime (R>2); CPM=Cefepime (R>4); ETP=Ertapenem (R>1); MEM=Meropenem (R>8); AMI=Amikacin (R>16); GEN=Gentamicin (>4); CIP=Ciprofloxacin (R>1); NFT=Nitrofurantoin (R>64); SXT=Sulphamethoxazole-trimethoprim (R>4); TGC=Tigecycline (R>2);CST= Colistin (R>2)

[2] There is no EUCAST breakpoint for cefoxitin, hence the CLSI breakpoint was used for cefoxitin.

Conclusion: Multi-drug resistant bla_{NDM-1}- and/or bla_{GES-5}-expressing clinical Enterobacteriaceae isolates are present in private hospitals in Durban, South Africa, causing clonal and multi-clonal outbreaks. An unprecedented multi-clonal NDM-1 outbreak in *K. pneumoniae* ST101 and *S. marcescens* SA1 clone is reported, linked to pNDM-HK-like and pRJF866-like plasmids. Colistin and tigecycline resistant Enterobacteriaceae co-expressing β-lactamase, *arr, fosA, rmtC*, and *qnr* genes are present in South Africa and is indicative of our failing antibiotic reserves, notably those used in combination therapy for carbapenem-resistant infections.

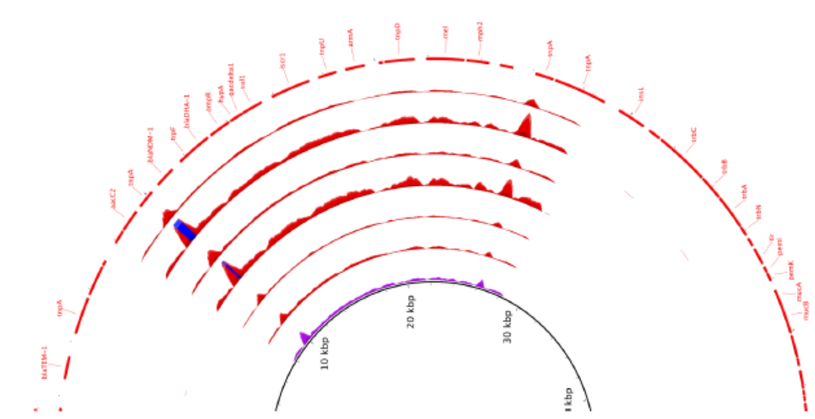

Figure 1B: BLAST comparison of the bla_{NDM-1} positive *K.pneumoniae* isolates (UNN39_S3, UNN40_S4, UNN46_S10, 12_S5, 32_S15, 53_S27 and 13_S6) using pNDM-HK (GenBank no. HQ451074) as a reference plasmid. (A Tn3-like structure homologous to the corresponding structure of plasmid pNDM-HK was present in all isolates, but the remaining plasmid DNA was not present in the isolates.)

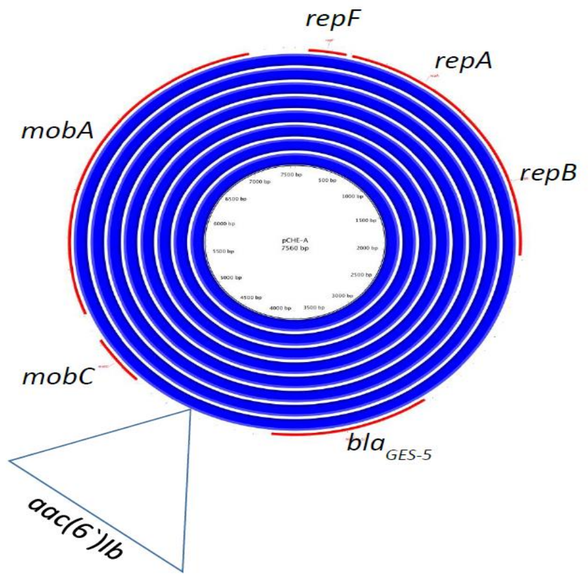

Figure 1A: BLAST comparisons performed by BRIG. The wgs of the bla_{GES-5} *K.pneumoniae* isolates (15_S8, 18_S10, 34_S16, 35_S17, 36_S18, 38_S19, 52_S36) were compared to pCHE-A (EU266532) as reference plasmid. (All of plasmid encoded DNA sequence was present in the isolates, and for most, the plasmid sequence circularized.) Insertion site of a *aac(6`)Ib* gene detected in all the isolates by direct sequence alignment with pCHE-A, are marked in the figure.

References

1. Stein C, Makarewicz O, Bohnert JA, Pfeifer Y, Kesselmeier M, Hagel S, et al. Three Dimensional Checkerboard Synergy Analysis of Colistin, Meropenem, Tigecycline against Multidrug-Resistant Clinical Klebsiella pneumonia Isolates. PLoS One 2015;10:e0126479. doi:10.1371/journal.pone.0126479.
2. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012;67:2640–4. doi:10.1093/jac/dks261.
3. Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, et al. ARG-ANNOT, a New Bioinformatic Tool To Discover Antibiotic Resistance Genes in Bacterial Genomes. Antimicrob Agents Chemother 2014;58:212–20. doi:10.1128/aac.01310-13.
4. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res 2014;42:D206–14. doi:10.1093/nar/gkt1226.
5. Alikhan N-F, Petty NK, Ben Zakour NL, Beatson SA. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 2011;12:402. doi:10.1186/1471-2164-12-402.

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