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Short communication

In vitro susceptibility of multi-drug resistant *Pseudomonas aeruginosa* and extended-spectrum β-lactamase–producing *Klebsiella pneumoniae* isolated from clinical specimens at Bugando Medical Centre, Tanzania to Piperacillin-Tazobactam

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Abstract: *Pseudomonas* spp. and *Klebsiella pneumoniae* are common causes of serious health care associated infections (HCAIs) worldwide. The treatment options for infections caused by multi-drug resistant (MDR) organisms are limited to tigecycline and carbapenems. A total of 172 isolates of multi-drug resistant *Pseudomonas* spp and extended-spectrum β- (ESBL) producing *Klebsiella pneumoniae* isolated from clinical specimens at the Bugando Medical Centre were tested for their *in vitro* susceptibility to piperacillin-tazobactam 100/10µg using disc diffusion test as recommended by Clinical Laboratory Standard Institute (CLSI). Out of 59 multi-drug resistant *Pseudomonas* spp, 54 (92.0%) were susceptible to piperacillin-tazobactam while of 113 ESBL producing *Klebsiella pneumoniae,* 55 (48.7%) were susceptible to piperacillin-tazobactam 100/10µg. Also, 20 (34.0%) of the *Pseudomonas* spp were both ESBL producers and susceptible to piperacillin-tazobactam 100/10µg. A significant proportion of *Pseudomonas* spp isolates from clinical specimens in our setting are susceptible to piperacillin/tazobactam. This study shows that piperacillin-tazobactam offer a better option to clinicians for the treatment of health care associated infections due to *Pseudomonas* spp. and ESBL producing *Klebsiella pneumoniae* in our setting and other health facilities where these organisms are of significance.

Keywords: multi-drug resistant, *Pseudomonas aeruginosa, Klebsiella pneumonia,* Piperacillin-Tazobactam, Tanzania

Pseudomonas aeruginosa and *Klebsiella pneumoniae* are bacteria commonly associated with serious infections in hospitalized patients worldwide. Treatment of infections caused by these bacteria is a challenge as they possess multiple resistance mechanisms including β-lactamases production, efflux pumps and impermeable outer membrane (Livermore, 2002). Despite the improved antibiotic therapy, infections due to multi-drug resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are still associated with increased morbidity and mortality in health care associated settings (Micek *et al.,* 2005) . Therapeutic options of these pathogens are limited to carbapenem and tigecycline which are too expensive and most often unavailable in developing countries. This study was conducted to determine *in vitro* susceptibility pattern of multi-drug resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* to piperacillin/tazobactam, the antibiotic that has been introduced to the market in Tanzania recently. The information generated here is crucial to clinicians so that they can make informed decision when they are prescribing antibiotics to treat these multi-drug resistant organisms.

This was a laboratory based study conducted at Bugando Medical Centre (BMC) in Mwanza, Tanzania. BMC is a 1,000 bed capacity referral and teaching hospital serving a population of about 13 million people in the Lake Victoria zone of Tanzania. A total of 172 multi- drug resistant *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* from clinical specimens were involved in the study. These isolates were identified as described previously (Mshana *et al*., 2009) and ESBL productions were confirmed using disc approximation test method. Susceptibility pattern to piperacillin-tazobactam

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100/10µg (Oxoid, UK) was done using disc diffusion method as recommended by CLSI. Briefly, colonies from overnight pure culture were suspended in sterile normal saline to 0.5 McFarland and inoculated on Muller Hinton agar (Oxoid, UK). Using sterile forceps piperacillin-tazobactam 100/10µg discs was placed on the media and plates incubated at 37°C for 18 hours. Interpretation was done according to Clinical Laboratory Standard Institute guidelines whereby the diameter of >18mm was recorded as sensitive and ≤18mm was recorded as resistance for *Pseudomonas aeruginosa* while for *Klebsiella pneumoniae* the diameter of ≥21mm was considered as sensitive, 18-20mm as intermediate while ≤17mm as resistance (Figure 1).

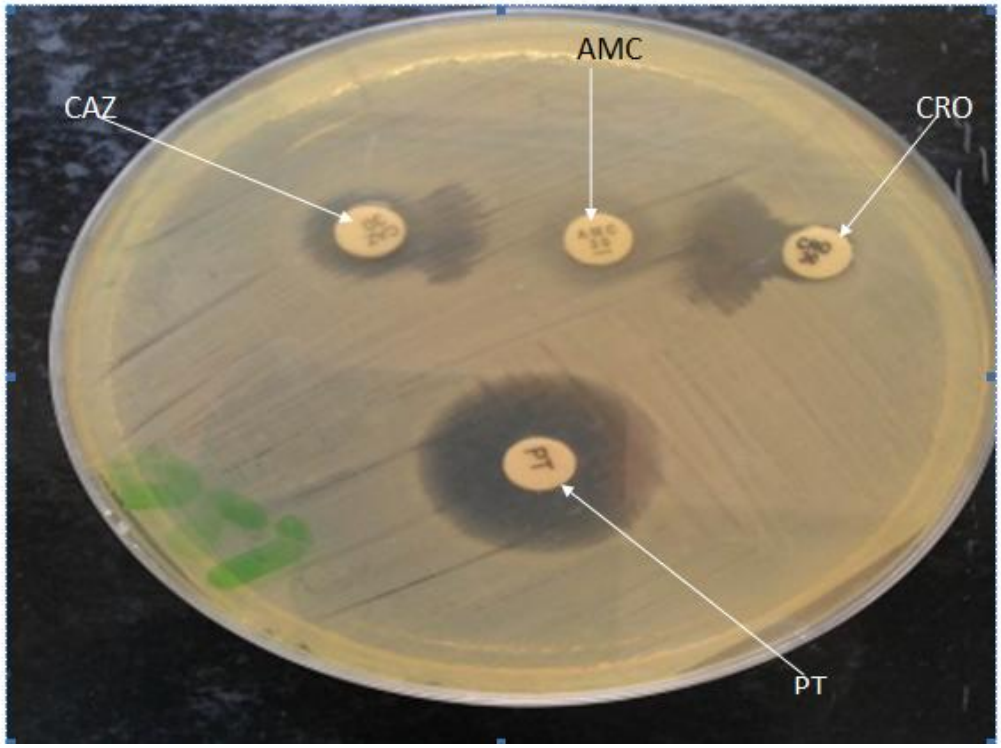

Figure 1: Disk approximation test showing the phenotypic phenomena of ESBL producing *Pseudomonas* spp and Piperacillin-tazobactam (PT)
Key: CAZ = Ceftazidime; AMC = Amoxillin/Clavulanic acid; CRO = Ceftriaxone

We evaluated the antimicrobial susceptibility pattern of 133 ESBL producing [*Pseudomonas aeruginosa* (20) and *Klebsiella pneumoniae* (113)] and 39 multi-drug resistant non-ESBL producing *Pseudomonas* spp. Majority of these isolates were from blood (76), while others were from pus swabs (74), urine (20), and aspirate (2) (Table 1). Out of 172 multi-drug resistant isolates in the study 113 (65.7%) were *Klebsiella pneumoniae* whereas among 59 *Pseudomonas aeruginosa* isolates, 20 (33.9%) were found to be ESBL producers.

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Table1: Different clinical specimen used in the study in percentages

Clinical specimen	Frequency	Percent
Blood	76	44.19
Pus swab	74	43.02
Urine	20	11.63
Aspirate	2	1.16
Total	172	100

Carbapenem has been preferred as the drug of choice for treatment of multi drug resistant bacteria (Paterson *et al.*, 2001), this class of antibiotics are unavailable and too expensive to most of Tanzanians. In Tanzania high mortality has been observed with infections associated with ESBL producing gram negative bacteria (Kayange *et al.,* 2010). In our setting, high prevalence of health care associated infections due to ESBL producing *Klebsiella pneumoniae* have been observed in surgical wards and neonatal units (Mshana *et al.*, 2013). *Pseudomonas spp* also have been found to be common causes of wound infections in our setting (Mbunda *et al.*, 2012). In the present study majority of *Pseudomonas* spp were from wound infections out of which 33.9% of them were found to produce ESBL. Compared to previous findings (Mshana *et al.,* 2009) there is increase in ESBL producing *Pseudomonas spp* in our setting, however higher rates than this have been reported elsewhere (Begum *et al.*, 2013).

Table2: Susceptibility pattern of multi drug resistance *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* to Piperacillin/Tazobactam

Isolate species	Interpretation	Frequency	Percentage
Pseudomonas aeruginosa	Resistant(R)	5	8.47
	Sensitive(S)	54	91.83
	Total	59	100.00
Klebsiella pneumoniae	Resistant(R)	10	8.85
	Intermediate(I)	48	42.48
	Sensitive(S)	55	48.67
	Total	113	100.00

Ninety two percent of *Pseudomonas aeruginosa* isolates (54/59) were susceptible to piperacillin-tazobactam 100/10µg while out of 113 ESBL producing *Klebsiella pneumoniae* isolates, 55 (48.7%) were susceptible to piperacillin-tazobactam 100/10µg ($p < 0.0001$; X^2 30.67). Similar findings were observed previously in Malaysian hospital (Pathmanathan *et al.*, 2009). Our results and other similar studies suggest that piperacillin-tazobactam may be good available alternative antibiotics to carbapenems for infections due *Pseudomonas* spp and to about 50% of ESBL producing *Klebsiella pneumoniae* in areas where *in vitro* susceptibility data is not availability (Ejaz *et al.*, 2011; Seni *et al.*, 2013). In general, the sensitivity rates of multi-resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* to Piperacillin-tazobactam in our setting is higher than that of cefepime (Mshana *et al.*, 2011)

A significant proportion of *Pseudomonas aeruginosa* isolates in our setting are susceptible to piperacillin-tazobactam. It is essential to understand local susceptibility patterns in order to optimize treatment of infectious diseases; hence, more of these studies need to be carried out. This study shows that piperacillin-tazobactam in the availability of susceptibility results offer a better option to clinicians for the treatment of health care associated infections due to *Pseudomonas* spp. and ESBL producing *Klebsiella pneumoniae* where these organisms are of public health significance.

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References

Begum, S., Salam, M.A., Alam, K.F., Begum, N., Hassan, P. & Haq, J.A. (2013) Detection of extended spectrum β-lactamase in Pseudomonas spp. isolated from two tertiary care hospitals in Bangladesh. *BMC Research Notes* 6: 7.

Kayange, N., Kamugisha, E., Jeremiah, S., Mwizamholya, D.L. & Mshana, S.E. (2010) Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza- Tanzania. *BMC Pediatrics* 10**,** 39.

Livermore, D.M. (2002) Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? *Clinical Infectious Disease* 34**,** 634-640.

Mbunda, F., Mchembe, M., Chalya, P., Rambau, P., Mshana, S., Kidenya, B. & Gilyoma, J. (2012) Experiences with surgical treatment of chronic lower limb ulcers at a tertiary hospital in northwestern Tanzania: A prospective review of 300 cases. *BMC Dermatology* 12**,** 17.

Micek, S.T., Lloyd, A.E., Ritchie, D.J., Reichley, R.M., Fraser, V.J. & Kollef, M.H. (2005) *Pseudomonas aeruginosa* bloodstream infection: importance of appropriate initial antimicrobial treatment. *Antimicrobial Agents and Chemotherapy* 49**,** 1306-1311.

Mshana, S.E., Hain, T., Domann, E., Lyamuya, E.F., Chakraborty, T. & Imirzalioglu, C. (2013) Predominance of *Klebsiella pneumoniae* ST14 carrying CTX-M-15 causing neonatal sepsis in Tanzania. *BMC Infectious Diseases* 13: 466.

Mshana, S.E., Kamugisha, E., Mirambo, M., Chakraborty, T. & Lyamuya,E. (2009) Prevalence of multiresistant Gram-negative organisms in a tertiary hospital in Mwanza, Tanzania. *BMC Research Notes* 2.

Mshana, S.E., Kidenya, B.R. & Kataraihya, J.B. (2011) *In vitro* activity of cefepime against extended spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* from clinical specimens at Bugando Medical Centre, Mwanza. Tanzania. *Tanzania Journal of Health Research* 13 (4).

Pathmanathan, S.G., Samat, N.A. & Mohamed, R. (2009) Antimicrobial susceptibility of clinical isolates of Pseudomonas aeruginosa from a Malaysian Hospital. *The Malaysian Journal of Medical Sciences* 16**,** 27.