Original Research Article

In Vitro Assessment of Fosfomycin: A Beacon of Hope in Drug Resistant Organisms Causing Urinary Tract Infections

ABSTRACT:

Urinary tract infections (UTI) are the most common bacterial infections affecting humans.. Fosfomycin has been approved for use in uncomplicated UTI caused by *E. coli* and *Enterococcus*. However, data regarding sensitivity of organisms causing hospital acquired or complicated UTI is scarce worldwide. We aimed to determine the in vitro sensitivity of drug resistant organisms causing hospital acquired and complicated UTI towards fosfomycin. Over a 6 month period, urine samples were processed as per standard microbiological protocols. Fosfomycin sensitivity was tested by disc diffusion assay and minimum inhibitory concentration (MIC) was determined by E test method. A total of 248` organisms causing hospital acquired and/or complicated UTI were isolated of which *E. coli* 88(35.48%) was most common followed by *K. pneumoniae* 78(31.45%) and *P. aeruginosa* 64(25.80%). Of 248, 92.74% (230/248) isolates were sensitive to fosfomycin. All the *E. coli* isolates were sensitive to fosfomycin with a low MIC (range 0.064-16 mg/L) while 97.43% (76/78) of the *K. pneumoniae* and 71.87% (46/64) *P. aeruginosa* of isolates were sensitive with a higher MIC (range 0.5-32 mg/L and 6-64mg/L respectively). Fosfomycin MIC geometric mean among *E. coli*, *K. pneumoniae* and *P. aeruginosa* was; 1.05, 7.19 and 19.61 mg/L respectively. *K. pneumoniae* and *P. aeruginosa* showed a significantly higher geometric mean MIC compare to *E. coli* (P <0.0001).This study suggests that fosfomycin has the potential to replace the parenteral antibiotics for treating complicated or hospital acquired lower UTI especially in case of Enterobacteriaceae.

Keywords: Antimicrobial resistance, Urinary Tract Infections, Fosfomycin

1. INTRODUCTION:

Urinary tract infections (UTI) are the most common bacterial infections affecting humans[1]. They can be uncomplicated or complicated urinary tract infections (cUTIs), the latter occurring in patients with anatomic or functional abnormalities of the urinary tract or in those with significant comorbidities[2]. UTI can also be classified as community acquired or hospital acquired. Majority of UTIs can be attributed to *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus saprophyticus* in case of community acquired UTI while in case of hospital acquired UTI, more unusual micro-organisms such as *Staphylococcus aureus, Enterococcus spp, Proteus spp, Pseudomonas aeruginosa, Acinetobacter spp*, and *Candida spp*[1-4] are implicated with a higher likelihood of antimicrobial resistance in addition, reflecting the attributes of the hospital flora. In case of cUTI, there is a higher risk of relapse, recurrence and mortality compared with uncomplicated UTIs[2] and more often than not, treatment guideline options have to be tailored to individual circumstances.

With increasing reports of ESBL, AmpC and carbapenemases producing bacteria causing UTI[3, 5], the decision to start the correct antibiotic at the appropriate time is becoming a challenge for the practicing physician. Current recommendations of the Infectious Diseases Society of America (IDSA) as well as European Society for Clinical Microbiology and Infectious Diseases (ESCMID) recommends fosfomycin and as one of the first-line agents to treat acute uncomplicated UTIs in adult females[6].

Fosfomycin is a phosphonic acid derivative, available as an oral formulation of fosfomycin tromethamine, a 5.7-gram powder sachet[7]. Approximately 40% of an oral dose of fosfomycin is excreted unchanged in urine following oral administration of a single dose. The mean urine fosfomycin concentration is 706 mg/L and declines to 10 mg/L in samples collected 72h after the dose[3]. It exerts its action by irreversible inhibition of MurA (UDP-N-acetylglucosamine-3-enolpyruvyl transferase), the cytosolic enzyme responsible for the first step in the peptidoglycan biosynthesis pathway that produces UDP-N-acetylmuramic acid[7]. This is a unique mechanism of action compared to other cell wall inhibitors suggesting that cross resistance between these drugs is unlikely. Fosfomycin enters the cytosol either by the glucose-6-phosphate- (G6P) inducible hexose-monophosphate transport (UhpT) system which is the primary portal, or less efficiently via the glycerol-3-phosphate) uptake (GlpT) system. Most Enterobacteriaceae, *Enterococcus* spp, and *Staphylococcus* spp. possess the UhpT transport system in their cell membrane[1].

The efficiency of fosfomycin against *E. coli* and *Enterococcus*, organisms that commonly cause community acquired UTI is well established. However, the data regarding the sensitivity of complicated UTI or hospital acquired organisms towards fosfomycin is lacking not only from India but also worldwide. Hence, we attempted to study the organisms causing hospital acquired and complicated UTI in our hospital and establish their in vitro sensitivity towards fosfomycin as a first step towards the use of fosfomycin for in patient treatment of UTI.

2. MATERIALS AND METHODS:

This prospective study was conducted in the Department of Microbiology of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India from 1 April 2016 to 30 September 2016. We studied drug resistant isolates[8] of gram negative bacteria from urinary

samples obtained from complicated[9] or hospital acquired[10] UTI. Identification of bacterial growth was done using standard techniques[11] and confirmed by an automated identification system(BD Phoenix™ 100).

Antimicrobial susceptibility testing was done on Mueller Hinton media by Kirby Bauer's disc diffusion method using discs obtained from Thermo Scientific™(Oxoid™) India Pvt Ltd, Mumbai, India. In addition, MIC of fosfomycin was determined by E test strips obtained from Hi-Media Laboratories, Mumbai, India. The drug resistant isolates were classified as multidrug resistant(MDR), extensively drug resistant(XDR) and pan drug resistant(PDR) according to standard definition[8]. Interpretation was done according to Clinical and Laboratory Standards Institute(CLSI) guidelines[12]. In case of fosfomycin, sensitivity was also compared with European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines[13].

Geometric MIC was calculated by Graph Pad Prism Software and one-way analysis of variance (ANOVA) with two sided Bonferroni multiple comparison test was performed for assessment of significance. Statistical significance was defined when p value was < 0.05.

3. RESULTS:

A total of 24,328 urine samples with clinical suspicion of UTI were processed. Of these, 2,510(10.32%) showed significant growth of pathogens. Majority were gram negative bacilli 1,720(68.52%) and among them 248(14.41%) were drug resistant (including MDR, XDR and PDR) according to the definition and were thus included for further study. Of total 248, 134(54.03%) were from patients previously on antibiotics or with abnormalities of urinary tract or significant co-morbidities and were thus deemed complicated UTI while remaining 114(45.97%) cases were acquired after 48 hours of hospitalization and were deemed hospital acquired UTI.

Of 248 multidrug resistant organisms, the distribution of organisms was; 88(35.48%) *Escherichia coli*, 78(31.45%) *Klebsiella pneumoniae*, 64(25.81%) *Pseudomonas aeruginosa*, *Morganella morganii* 6(2.42%), *Citrobacter freundii* 6(2.42%), *Acinetobacter baumannii* 4(1.61%) and *Providencia rettgeri* 2(0.81%)[Fig 1].

Among 248 isolates, 92.74% (230/248) were sensitive to fosfomycin [Fig 2]. Analysis of individual isolates reveals that all *E. coli* were sensitive to fosfomycin. Colistin was the other drug to which 97.73% (86/88) isolates of *E. coli* were sensitive followed by nitrofurantoin 52.27%(46/88).However, 97.43%(76/78) *K. pneumoniae*, isolates were sensitive to fosfomycin followed by colistin 92.31% (72/78).In addition, 71.87% (46/64) *P. aeruginosa* isolates, were sensitive to fosfomycin while a higher number 52(81.25%) were sensitive to colistin. Among the other gram negative bacilli isolates, only 2 isolates of *A. baumannii* and 2 isolates of *M. morgannii* were resistant to fosfomycin. All other isolates were sensitive to fosfomycin[Fig 2]. Comparison of the sensitivity of various drugs in contrast with fosfomycin has been depicted in fig. 3.

On comparison of resistance rates when interpretation was done according to CLSI and EUCAST, the number of isolates resistant to *E. coli* and *K. pneumoniae* did not change. However, as 6 isolates of *P. aeruginosa* had an MIC of 64 mg/L which is resistant according to EUCAST but sensitive according to CLSI, the resistance rate for *P. aeruginosa* rose to 24(37.5%) by EUCAST from 18(28.12%) by CLSI[Table 1].

Analysis of the range of MIC of the different organisms reveals an interesting pattern[Figure 4]. All the *E. coli* isolates in our study were not only sensitive to fosfomycin but also had very low MICs with range 0.064-16 mg/L and geometric mean(GM) 1.05 mg/L. On the other hand, sensitive *K. pneumoniae* strains had MIC in the range of 4-32 mg/L with GM of 7.19 mg/L while the sensitive isolates of *P. aeruginosa* had an MIC range of 6-64 mg/L with GM of 19.61 mg/L. This difference in the geometric mean of *K. pneumoniae* and *P. aeruginosa*

from *E. coli* was statistically significant with p <0.001[Fig 4]. The MIC50 and MIC90 of these organisms have been depicted in table 1.

4. DISCUSSION:

Fosfomycin represents a potentially reliable treatment option for UTIs, particularly the drug-resistant variety[14]. However, significant discrepancies occur between broth and agar dilution methods for determining MIC of fosfomycin and so far, agar dilution is the only approved fosfomycin MIC susceptibility testing method[1]. As most automated systems for antimicrobial susceptibility testing are microdilution-based methods, resistance to fosfomycin may be overestimated in laboratories employing such systems[15]. Hence, we attempted to study the in vitro susceptibility of drug resistant gram negative bacilli causing UTI by disc diffusion and E test method which are more commonly available and practiced in our country.

In this study, the most common drug resistant gram negative pathogens causing UTI were *E. coli* and *K. pneumoniae* followed by *P. aeruginosa* similar to many other studies[16-19]. Overall 7.26% of the isolates were resistant to fosfomycin similar to other studies such as Seroy et al(6%)[3], Demir et al(6.1%)[17] and Hirsch et al (5.6%)[20], but much less than that reported by Linsenmeyer et al(21.6%)[16] and Kaase et al(28%)[21].

All drug resistant *E. coli* isolates in our study were sensitive to fosfomycin with 100% of the isolates having and MIC of less than or equal to 16. This is similar to other studies[4, 15, 17-20, 22, 23]. In our study, 97.4% of the *K. pneumoniae* isolates were sensitive to fosomycin. This is similar to the study by Falagas et al[7], Demir et al[17], Perdigao-Neto et al[23] but in contrast to the study by Liu HY et al[4], Linsenmeyer et al[16], Livermore et al[24] and

Chitra et al[25] who found only 42%, 54%, 52%, and 64% of their *K. pneumonaie* isolates sensitive to fosfomycin respectively. Also, the MIC of *K. pneumoniae* was considerably more than that of *E coli* and this has been demonstrated in studies by other researchers as well[7, 21, 24, 26].

In our study only 9(28.12%) of the 32 *P. aeruginosa* isolates were resistant to fosfomycin. Although this is clearly in excess of the resistance rates in Enterobacteriaceae, it is still much less than that reported by other researchers[17, 27, 28]. It is also in contrast to the study by Sultan et al[18] and Perdigao-Neto et al[23] in which 100% *P aeruginosa* isolates were sensitive to fosfomycin. The MICs of most *P. aeruginosa* isolates in our study was uncomfortably close the the breakpoint of 64ug/ml so empirical use of fosfomycin against *P. aeruginosa* would not be reasonable. Another interesting finding in our study was that although E test has been recently reported to perform poorly for *P. aeruginosa*[20, 23, 29] but in our study, there was absolute correlation between E test and disk diffusion.

As demonstrated by the geometric mean, there is a clear gradation of the MIC range with lowest values seen in *E coli* and significantly($p < 0.001$) higher values seen in *Klebsiella* and *Pseudomonas* progressively and this has also been demonstrated by other studies[1,23]. Thus the activity of fosfomycin may not be as reliable if used empirically in the absence of susceptibility testing for *P. aeruginosa*[20]. On the other hand, even with high MIC we cannot predict without clinical trials that therapeutic failure is the predictable outcome[30].

The CLSI has established that for *E. coli* and *Enterococcus*, susceptibility to fosfomycin is defined as an MIC ≤64 mg/L but MIC breakpoints are lacking for other gram-negative organisms[12]. EUCAST defines a fosfomycin MIC ≤32 mg/L as susceptible for urinary Enterobacteriaceae and *Pseudomonas* isolates[13]. This discrepancy makes interpretation and

comparison of results from different studies difficult[7]. However, in our study, the resistance rate of *E. coli* and *K. pneumoniae* did not vary between the two methods although the resistance rate of *P. aeruginosa* increased to 12(37.5%) when interpreted by EUCAST from 9(28.12%) when interpreted by CLSI guidelines. In case of *E. coli* and *K. pneumoniae*, other researchers have also reported minimal variation in resistance rates by the two methods[1,22] while significant variation in the resistance rate of *P. aeruginosa* has also been reported[23]. However, an Indian study by Chitra C et al has reported significant variation even in the resistance rate of *K. pneumoniae* on interpretation by EUCAST(45%) and CLSI(13%) method[25].

In our study, Colistin was the antimicrobial most sensitive against the isolates after fosfomycin and in case of *P. aeruginosa*, it was even slightly better than fosfomycin. However, Colistin is not a practical choice for UTI as nephrotoxicity is one of its prominent side effects and dose adjustment is required in case of renal impairment[31]. Similarly, other parenteral alternatives such as carbapenems, aminoglycosides and piperacillin-tazobactam performed poorly against these isolates.

Oral antibiotics which are advised as first line against UTI such as nitrofurantoin, cotrimoxazole and fluoroquinolones were also widely resistant and thus of no practical use for these isolates. This has been reported by many other researchers as well[2, 4, 16, 17, 19] This may be due to the widespread misuse of these drugs for every outpatient indication and lack of implementation of adequate guidelines for prescribing antibiotics. A notable exception is nitrofurantoin in the case of drug resistant *E. Coli,* 46(52.3%) of our 88 *E. coli* showed sensitivity indicating that this antibiotic still has some role in UTI caused by *E. coli*[16, 19].

Looking at the pattern of sensitivity of these drug resistant isolates towards fosfomycin as

compared to other commonly used antibiotics, it would not be unreasonable to suggest that fosfomycin has the potential to replace the parenteral antibiotics for treating complicated or hospital acquired lower UTI especially in case of Enterobacteriaceae. The benefits of such a shift would not only be the use of an oral antibiotic with an excellent safety profile achieving high concentration in the urine but also preventing the emergence of resistant micro-organisms while reserving the parenteral antibiotics for a more aggressive systemic infection. However, such a decision will need the backing of clinical trials to ascertain its rationality.

5. CONCLUSION:

The satisfaction of improved patient survival is often threatened by the development of health care associated infections, the most common of which is UTI often caused by a drug resistant bacteria. As we stare down the barrel of dwindling treatment options, with their own unacceptable toxicities, we are forced to look back at the antimicrobials we discarded and re-think our management strategies. Our study suggests that fosfomycin is one such drug which is safe, with minimal adverse effects, achieves high concentration in urine, has low levels of non transmissible resistance among bacteria and thus can be used in cases of hospital acquired or complicated UTIs on the basis of a sound test for susceptibility.

6. CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

7. REFERENCES:

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FIGURES AND TABLES:

Figure 1: Distribution of organisms isolated and their resistance types (MDR, XDR or PDR)

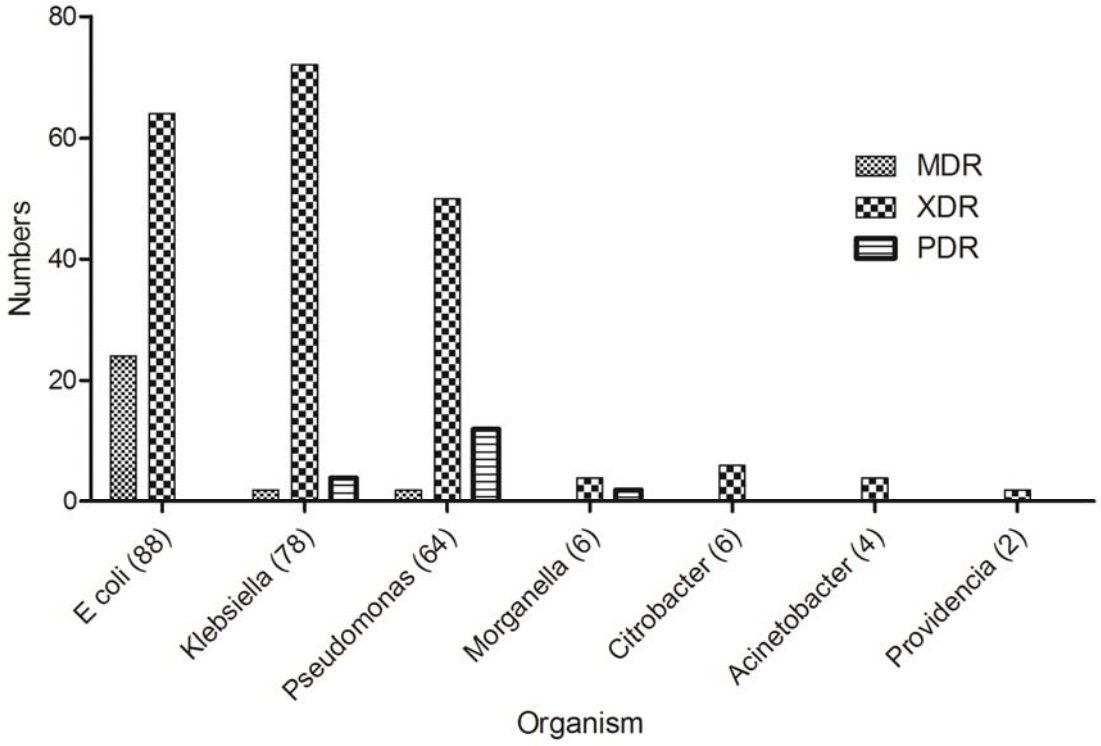

Figure 2: Sensitivity of the isolates against fosfomycin

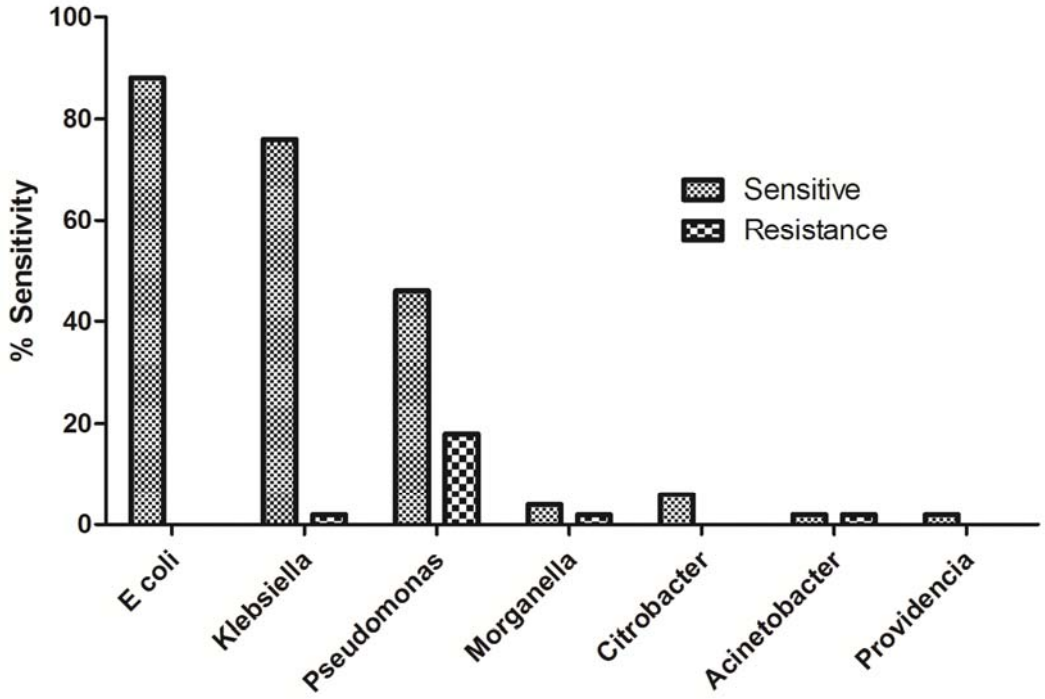

Figure 3: Sensitivity of E coli, K pneumoniae and P aeruginosa isolates to various antibiotics in comparision to fosfomycin

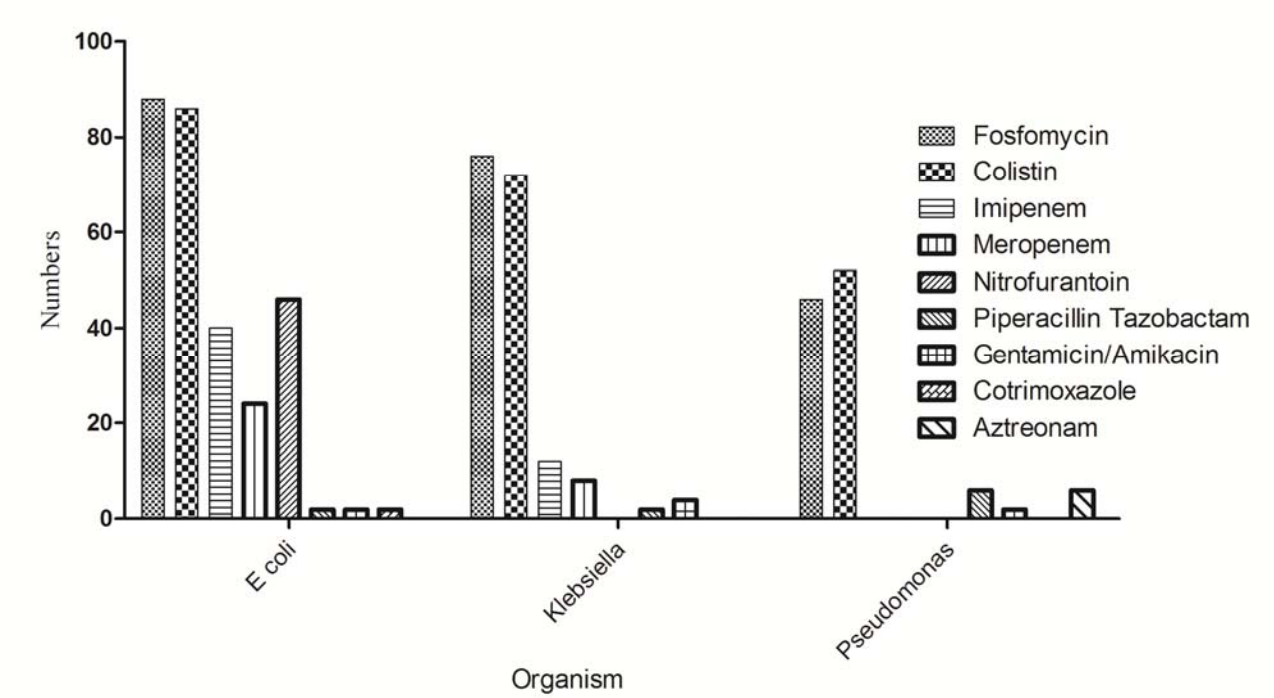

Table 1: Interpretation of sensitivity of the drug resistant isolates to fosfomycin by CLSI and EUCAST criteria

Organism Total (n)	n(%) CLSI			n(%) EUCAST			
	S ≤ 64	S ≤ 32	S ≤ 32	S ≤ 32	R >32	MIC_{50}	MIC_{90}
E coli(88)	88 (100%)	88 (100%)	88 (100%)	88 (100%)	0 (0%)	1	8
K pneumoniae(78)	76 (97.4%)	76 (97.4%)	76 (97.4%)	76 (97.4%)	2 (2.6%)	8	24
P aeruginosa(64)	46 (71.9%)	40 (62.5%)	40 (62.5%)	40 (62.5%)	24 (37.50%)	32	64

370 Figure 4: Range of MIC of E coli, K pneumoniae and P aeruginosa isolated interpreted

371 according to CLSI

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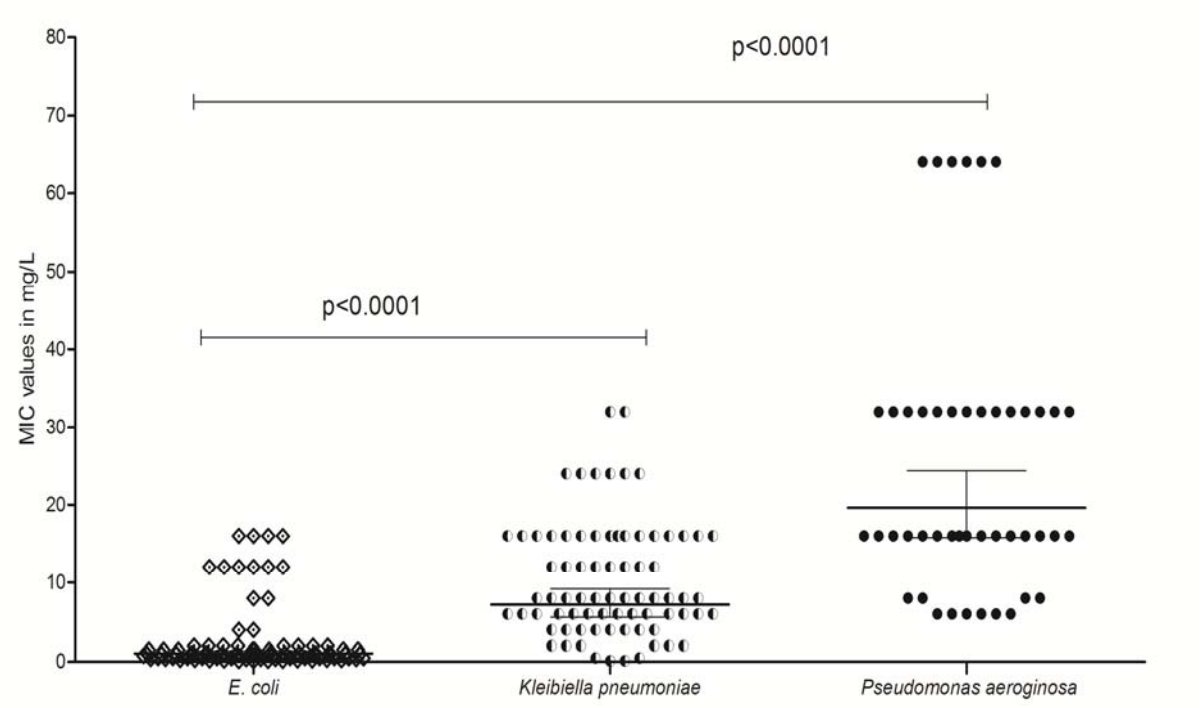

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