Direct reporting cefazolin from VITEK® 2 for *E. coli*, *K. pneumoniae* and *P. mirabilis* isolated from urine cultures using new CLSI interpretations

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Abstract

Background: In recent years, Clinical and Laboratory Standard Institute (CLSI) has recommended a series breakpoint changes for cefazolin, including testing it as a surrogate agent for oral cephalosporins for treating uncomplicated urinary tract infections (uUTIs).

Objectives: This study was conducted to evaluate the feasibility of direct reporting the cefazolin results from VITEK® 2 for *E. coli*, *K. pneumoniae*, *K. oxytoca* and *P. mirabilis* isolated from patients with uUTIs using 2014 CLSI recommendation.

Material and Methods: Cefazolin susceptibility results of urine cultures of the above four species generated from January 1, 2013 December 31, 2013, using both GN AST card N208 on VITEK® 2 and cefazolin disk (gold standard) methods, were extracted from SoftMic Laboratory Information System and analyzed for their category agreement using 2014 CLSI interpretations.

Results: Cefazolin susceptibilities of 1969 urinary isolates (1869 patients) of *E. coli*, *K. pneumoniae*/*K. oxytoca* and *P. mirabilis* comparing their VITEK® 2 and disk test results, category agreement for cefazolin tested with both methods was 98%. The linear correlation between sensitive cefazolin and sensitive cephalothin MICs versus cefazolin zone diameters was good, with a predictive value of 99%.

Conclusion: It is acceptable to report cefazolin directly from VITEK® 2 for the named species from urine cultures. The susceptibility correlation among cefazolin, cephalothin and cefixime were excellent (excluded non-susceptible), further testing with individual oral cephalosporin agents, in this institute, may not be necessary.

Recommendation: Final report accompanied by a comment in accordance with 2014 CLSI guideline is recommended to provide therapeutic guidance to clinicians.

Key Word: Cefazolin, Urine Culture, CLSI, Interpretations, VITEK® 2.

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Introduction

Cefazolin is a first generation cephalosporin; it was released for clinical use in 1973. Its antibacterial spectrum covers mainly Gram positive bacteria and some *Enterobacteriaceae* - *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Proteus mirabilis*; however, it is ineffective against methicillin resistant *Staphylococcus aureus* (MRSA), extended- spectrum β-lactamase (ESBL)-producing Gram negative bacteria and anaerobic bacteria. Cefazolin is administered via intravenous and intramuscular routes, is widely distributed except the central nervous system, and renally cleared with $t_{1/2}$ of 2 hours. Clinically, cefazolin is primarily used to treat skin and soft tissue infections, intra-abdominal infections, uncomplicated urinary tract infections (uUTIs) and for surgical prophylaxis[1,2,3].

Over the years, CLSI has changed the interpretive criteria for cefazolin susceptibility breakpoints several times (Table 1)[4,5,6,7]. Prior to 2010, cefazolin susceptibility results for *Enterobacteriaceae* isolates from the urinary tract obtained from the automated VITEK® 2 system (BioMerieux, Montreal, Canada) could be reported, because they fell within the cefazolin testing and reporting range of VITEK® 2 (4 – 64 mg/L) as set up by its Advanced Expert System™ (AES, software version 06.01)[8]. In 2010, CLSI changed the MIC breakpoints for cefazolin to S: <= 1 mg/L; I: 2 mg/L; R: >= 4 mg/L and subsequently in 2011 to S: <= 2 mg/L; I: 4 mg/L; R: >= 8 mg/L for *Enterobacteriaceae* isolated from all sites, with the intention to eliminate need for ESBL screen and confirmatory tests when using revised breakpoints[5,6]. These changes have negated the previous direct reporting of cefazolin susceptibility from VITEK® 2 which usually uses the FDA test interpretation criteria (http://www.drugs.com/pro/cefazolin-injection.html) but often reported through the configured AES in VITEK® 2 (Table 1). Consequently, laboratories that used VITEK® 2 and reported cefazolin sensitivity had to perform a supplementary Kirby-Bauer (K-B) using the 2011 zone diameter interpretations; notably, this practice

was not possible in 2010 since there was no interpretations for cefazolin disk in 2010 CLSI (Table 1). The extra K-B test increased the workload for laboratories and incurred additional cost. The breakpoint changes introduced in 2010-2011 also reduced the clinical utility of narrow spectrum cephalosporins for the treatment of uUTIs[9]. Through the years, CLSI has recommended cephalothin testing as a surrogate for the oral cephalosporins cefadroxil, cefpodoxime, cephalexin, and loracarbef results for treating uUTIs[4,5,6,7,9].

In 2014, CLSI (M100-S24) added a new cefazolin surrogate test for uUTIs with two recommendations. First, testing of cefazolin is preferred to the testing of cephalothin for predicting sensitivity results of oral cephalosporins when used for therapy of uncomplicated UTIs. Second, the new cefazolin interpretive criteria are recommended as a surrogate test to predict results for oral cephalosporins cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime axetil, cephalexin and loracarbef (a carbacephem), when these agents are used to treat uUTIs due to *E. coli*, *K. pneumoniae* and *P. mirabilis*. However, cefazolin can only predict susceptibility to oral cephalosporins but not resistance, because cefazolin resistant strains can still be susceptible to cefdinir, cefpodoxime, and cefuroxime axetil. If required, cefazolin-resistant strains may be individually tested with these agents[7].

The aims of this study were to verify the feasibility of direct reporting the cefazolin surrogate test results from VITEK® 2 for *E. coli*, *K. pneumoniae*, *K. oxytoca* and *P. mirabilis* isolated from patients with uncomplicated UTIs. It also compared the susceptibility correlation among cefazolin, cephalothin and cefixime as a side-product of this study.

Materials and Methods

We analyzed our cefazolin susceptibility results generated from January 1, 2013 - December 31, 2013. Cefazolin susceptibility results were obtained using GN AST card N208 on VITEK® 2 (BioMerieux, software Version 06.01) and cefazolin disk (30µg) (BBL, Becton Dickson) on Muller Hinton media (BioMedia, Toronto, Canada). The quality controls of both testing systems were carried out in accordance with the manufacturer's instruction and CLSI guidelines[6,10,11]. The data included 1969 urinary clinical isolates from 1869 patients - *E. coli* (1550 isolates/1538 patients), *K. pneumoniae* & *K. oxytoca* (261 isolates/260 patients) and *P. mirabilis* (158 isolates/157 patients). Sometimes, more than one species was isolated from one urine specimen. Cefazolin MIC and Disk susceptibility data along with susceptibility of cephalothin and cefixime MICs (on the same VITEK® 2 N208 card) were retrieved from Soft Mic Laboratory Information System (Version 4.0.4, SCC Soft Computer, Clear water, USA). Duplicated strains per episode or admission were excluded unless changes in sensitivity of the same species were noted, the isolates reported as ESBL/AmpC-producers were excluded. Category agreements (S, I and R) between VITEK® 2 and K-B (gold standard) for the new 2014 CLSI M100-S24 urinary interpretations as well as of minor, major and very major errors were determined[12]. The verification was considered acceptable if the category agreement was >90% using VITEK® 2 cefazolin testing as compared to Kirby-Bauer confirmation[12]. Cephalothin, another surrogate test agent for uUTIs, see (Table 1), was also studied for its predictive susceptibility comparing to the sensitivity of cefazolin. The linearity of the correlation between MICs and zone diameters of cefazolin and susceptibility profiles of the studied organisms were also analyzed by WHONET 5.6 software[13], which uses 2014 CLSI M100-S24 interpretations and WHONET software is available from:
http//www.who.int/drugresistance/whonetsoftware/en.

Table 1: Changes in cefazolin and cephalothin interpretations made by CLSI (2009 - 2014)

Cefazolin interpretation	Susceptible		Intermediate		Resistance		Reference
	MIC[1)]	Disk[2)]	MIC	Disk	MIC	Disk	
2014 CLSI M100-S24* Uncomplicated UTI	<= 16	>= 15	NA	NA	>= 32	<= 14	7
2014 CLSI M100-S24 Surrogate for Oral Cephalosporins	<= 16	>= 15	NA	NA	>= 32	<= 14	7
2014 CLSI M100-S24 Systemic Infections	<= 2	>= 23	4	20 - 22	>= 8	<= 19	7
2011 CLSI M100-S21^	<= 2	>= 23	4	20 - 22	>= 8	<= 19	6
2010 CLSI M100-S20˜	<= 1	NA	2	NA	>= 4	NA	5
2009 CLSI M100-S19#	<= 8	>= 18	16	15 - 17	>= 32	<= 14	4
Cephalothin	**Susceptible**		**Intermediate**		**Resistance**		4-7
2009 – 2014 CLSIs	**MIC**	**Disk**	**MIC**	**Disk**	**MIC**	**Disk**	
Surrogate for uUTIs	<= 8	>=18	16	15 - 17	>= 32	<= 14	

[1)] MIC unit: mg/L; [2)] Kirby-Bauer Disk unit: zone diameters in millimeters (mm); NA, not available
* 2015-2016 CLSI cefazolin interpretations are the same as 2014 CLSI;
^2012-2013 CLSI cefazolin interpretations remained the same as 2011;
~ FDA test interpretative criteria for cefazolin is the same as of 2010 CLSI;
# VITEK® 2 Advanced Expert System (software version 06.01) shares the same cefazolin interpretations as 2009 CLSI's.

Table 2: Susceptibility Profiles of 1969 isolates of *Enterobacteriaceae*

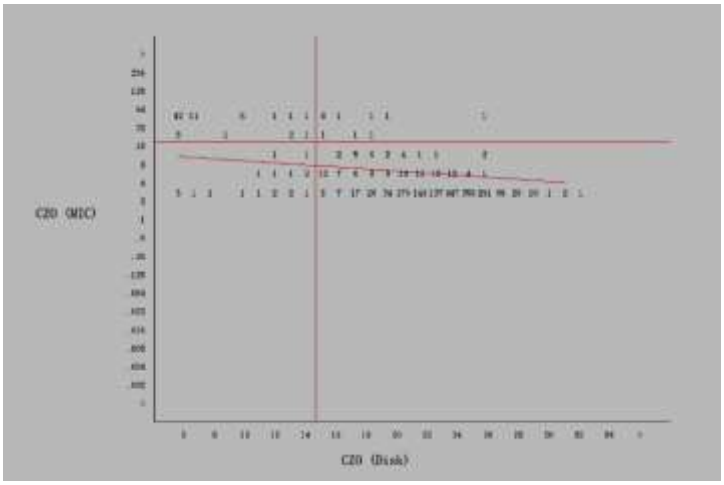
Organism	Numbers	S% CZO (DISK)	S% CZO(MIC)	S% CEP(MIC)	S% CFM(MIC)
E. coli	1550	96	96	65	95
K. pneumoniae	234	96	97	94	97
K. oxytoca	27	74	82	78	96
P. mirabilis	158	97	96	87	99

CZO: cefazolin; CEP=cephalothin; CFM: cefixime; DISK: disk diffusion method;
MIC: VITEK® 2 MIC test. S%: percent of susceptibility.

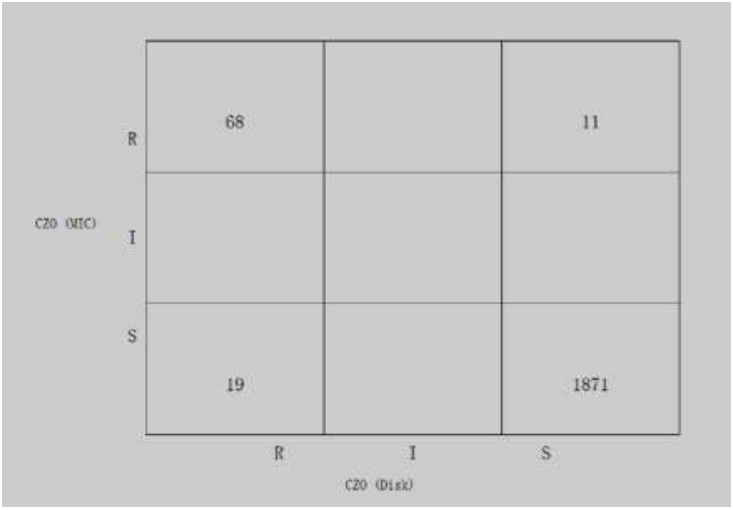
Table 3: Category disagreement between VITEK® 2 and Kirby-Bauer tests for cefazolin using 2014 CLSI M100-S24 Interpretations

	Minor Errors[a]	Major Errors[b]	Very Major Errors[c]	Category Disagreement (%)
E. coli (=1550)	0	8	13	1.4%
K. pneumoniae & *K. oxytoca* (=261)	0	0	4	1.5%
P. mirabilis (=158)	0	3	0	1.9%
Total=1969	0	11	17	1.4%

[a] The minor errors: the new system (VITEK® 2) indicates an intermediate result while the other system (K-B) indicates either a sensitive or resistant;
[b] Major errors: the new system (VITEK® 2) indicates a resistant while the reference system (K-B) indicates a susceptible response;
[c] Very major errors: the new system (VITEK® 2) indicates a susceptible while the reference system (K-B) indicates a resistant.



(A)



(B)

Fig. 1: The scatter plot of cefazolin MIC (Y axis) versus cefazolin disk tests (X axis) for1969 isolates of *E.coli, K. pneumoniae, K. oxytoca* and *P. mirabilis,* interpreted by 2014 CLSI (A): the Arabic numerals inside the plot are the numbers of isolates with the MICs equals to the zone sizes of cefazolin. The red line intersected with X axis represents the K-B test zone diameter > =15 mm which divides the sensitive (right side) from the resistant (left side). The red line intersected with Y axis represents the VITEK® 2 MIC breakpoint >=16 mg/L which divides the sensitive (below) from the resistant (above). Regression (the oblique red line) analysis showed: log2 (MIC) = 4.33 - 0.06disk, r = - 0.32. **Test interpretations between MIC versus Disk methods of cefazolin (B)**: the calculated sensitivity and specificity for CZO MIC as well as positive predictive value (PPV) and negative predictive value (NPV) for CZO MIC were: sensitivity (in detection of resistance) 78%, specificity 99%; PPV 86% and NPV 99%. CZO (MIC) indicates VITEK® 2 cefazolin MIC and CZO (Disk) is cefazolin Kirby-Bauer zone diameters in mm. The data was analyzed by WHONET 5.6 with 2014 CLSI M100-S24 interpretations.

Results and Discussion

The majority of the isolates were from adults (95%); the urine specimens' were collected from approximately 40% inpatients, 20% outpatients and 40% emergency. The susceptibility profiles of these organisms are shown in (Table 2). Most of the tested isolates were highly sensitive to cefazolin and cefixime due to ESBL- and AmpC-producing *Enterobacteriaceae* isolates were excluded from this study, except *K. oxytoca* which usually carries K1 β-lactamase[14] and only had 74% and 78% sensitivity to cefazolin and cephalothin, respectively. Additionally, cephalothin seemed to be more vulnerable to the resistance mechanism of *E. coli* and *P. mirabilis* with sensitivities of 65% and 87%, respectively (Table 2). Cefuroxime is not an agent in VITEK® 2 GN AST N208 card; therefore, there is no susceptibility information of this drug for this panel of bacteria. No third generation cephalosporins (ceftazidime, ceftriaxone and cefotaxime) were found to be resistant if cefazolin was sensitive in the 1969 tested organisms (data not shown). The verification results were presented in (Table 3); the category disagreement was less than 2% for each of the four *Enterobacteriaceae* species. VITEK® 2 AST N208 card has a MIC 4-64 mg/L reporting range for cefazolin, this includes MIC 16 mg/L =Intermediate, however, there is no "intermediate" category for the 2014 UTI breakpoints (Table 1). In this study, there were 23/25 "intermediate" VITEK® 2 results that fell into their K-B sensitive range, therefore, it was reasonable to report "I" as "S", in this situation; in accordance with 2014 CLSI recommendation of cefazolin MIC 16 mg/L = sensitive[7]. The linear correlation of cefazolin MIC versus cefazolin K-B zone diameter for the 1969 tested organisms was illustrated in (Fig. 1). Using the cefazolin K-B test as the gold standard and detection of resistance as the goal of the testing, the specificity of a sensitive cefazolin MIC was high (99%), i.e. the negative predictive value of cefazolin MIC was as high as 99%. Therefore, it is generally acceptable to report cefazolin directly from VITEK® 2 for the four species isolated from urine cultures using the 2014 CLSI interpretation. The laboratory has successfully implemented the direct reporting of cefazolin from VITEK® 2 susceptibility testing for *E. coli*, *K. pneumoniae* and *P. mirabilis* isolated from urinary cultures; for the cefazolin susceptible strain, a comment is added to the report: "Cefazolin sensitivity indicates susceptibility to oral agents including cefaclor, cefuroxime axetil, and cephalexin when used for therapy of uncomplicated UTIs due to *E. coli*, *K. pneumoniae*, and *P. mirabilis*." This new procedure saves extra

reagent cost > C$3000/year and decreases the final report turnaround time by 16-24 hours.

Since cephalothin as another surrogate for the oral cephalosporins[7] was also tested in GN AST N208 card, we took the opportunity to look at the correlation of cephalothin MIC versus cefazolin MIC or cefazolin Disk. Unlike cephalothin, cefazolin has no interpretation for intermediate MIC or zone sizes ranges (Table 1); therefore, it was difficult to include intermediate cephalothin results into either sensitive or resistant cefazolin MIC or Disk (K-B) categories for comparison of their category agreement. However, if following 2014 CLSI interpretation of cephalothin<= 8 mg/L equals to sensitive as the calling point to predict susceptibility to the oral cephalosporins[7], the two antibiotics had a good correlation for the subpopulation that was sensitive to both cephalothin and cefazolin (data not shown). Using cefazolin K-B test (disk zone sizes) as the gold standard, the sensitivity of cephalothin MIC (in detection of resistance) was 93% and 83% when comparing to cefazolin MIC and cefazolin disk, respectively; the specificity of a sensitive cephalothin MIC to predict a sensitive cefazolin MIC or disk was 95%, with the negative predictive value of cephalothin MIC was 99% for both cefazolin Disk and MIC, as calculated by WHONET 5.6 (data not shown).

There are some limitations of this study:(1) only about 8% of the tested strains belonged to the "challenging" strains; i.e. the strains had one MIC dilution difference on both sides of the breakpoint 16 mg/L by VITEK® 2 MIC method (MICs of 8, 16 and 32 mg/L); how robust the conclusion would have been if such strains are increased remains to be studied; also, given this was a retrospective study, we were unable to confirm whether there were technical errors leading to the category discrepancy results between cefazolin MIC and cefazolin disk methods, by a third method such as Etest;(2) several conditions may lead to misinterpretation of cefazolin sensitivity, such as equivocal resistance due to higher TEM-1/TEM-2/SHV-1 or inducible AmpC-producers[14,15], and how reliable K1 in *K. oxytoca* can be detected by VITEK® 2 susceptibility testing is unknown; therefore, if resistance is suspected, it is prudent to perform a K-B to confirm the susceptibility; (3) cefazolin should be interpreted as intrinsically resistant if the organism is an ESBL or AmpC-producing organism[14,15]; (4) *E. coli*, *K. pneumoniae* and *P. mirabilis* isolated from other body sites still require cefazolin K-B confirmatory testing[7]; (5) clinical correlation for the treatment of uncomplicated UTIs with oral cephalosporins whose susceptibilities are predicted by directly reporting cefazolin from VITEK® 2 remains to be further studied;(6) the results apply to uncomplicated UTIs only, and not for complicated infections such as catheter-associated UTIs or complicated pyelonephritis[16]. Therefore, clear communication between the laboratory and the treating clinician is essential.

Conclusions

In summary, it is generally acceptable and cost-effective to report cefazolin directly from VITEK® 2 for the four species of *Enterobacteriaceae* isolated from urine cultures using new 2014 CLSI interpretation. Final report must be accompanied by a comment in accordance with 2014 CLSI guideline, to provide therapeutic guidance to clinical staff. Most of the tested isolates were highly sensitive to cefazolin and cefixime because ESBL- and AmpC-producing *Enterobacteriaceae* were excluded; K1 β-lactamase-producing *K. oxytoca* were included but were less sensitive. The susceptibility correlation among cefazolin, cephalothin and cefixime were excellent (excluded non-susceptible), therefore, further testing with individual oral cephalosporin agents, in this institute, may not be necessary. Only 8% of organisms were near the breakpoint MIC =16 mg/L in this study ("challenging strains"), a further study with more challenge strains and a conventional cefazolin MIC method versus cefazolin disk diffusion test may be warranted to evaluate the linear relationship and category agreement of this subpopulation.

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Conflict of Interest: None

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References

1. Association CP. Cefazolin. In CPS 2015: Compendium of pharmaceuticals and specialties (50th ed.).-Ottawa, Canada.
2. Hansen E, Belden K, Silibovsky R, et al. 2014. Perioperative antibiotics. J Orthop Res. 32 Suppl 1:S31-59.
3. Schuetz AN, Brasso WB, Crandon JL, et al. 2013. Cefazolin as a class representative for oral cephalosporins and uncomplicated urinary tract infections caused by indicated Enterobacteriaceae. Diagn Microbiol Infect Dis.77:381-382.
4. CLSI. 2009. Performance standards for Antimicrobial susceptibility testing; twenty-fourth informational supplement. CLSI document M100-S19 Clinical and Laboratory Standards Institute, Wayne, PA., USA.
5. CLSI. 2010. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. CLSI document M100-S20 Clinical and Laboratory Standards Institute, Wayne, PA., USA.
6. CLSI. 2011. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. CLSI document M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA., USA.
7. CLSI. 2014. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. CLSI document M100-S24 Clinical and Laboratory Standards Institute,Wayne, PA., USA.
8. BioMerieux Inc. 2010. VITEK® 2 - technology software user manual 2010(May) 24482.
9. Turnidge JD. 2011. Cefazolin and enterobacteriaceae: rationale for revised susceptibility testing breakpoints. Clin Infect Dis. 52:917-924.
10. BioMerieux Inc. 2008. VITEK® 2 instrument user manual. 2008(June).510731-10EN1(24262).
11. BioMerieux Inc. 2010. VITEK® 2 systems product information 2010 (June).
12. Richard B. Clark MAL, Michael, J. Loeffelholz, and Robert J. Tibbetts. 2009. Cumitech 31A, Verification and Validation of Procedures in the Clinical Microbiology Laboratory. Coordinating ed., Susan E. Sharp. ASM Press, Washington, DC.
13. O'Brien TF, Stelling JM. 1995. WHONET: an information system for monitoring antimicrobial resistance. Emerg Infect Dis.1: 66.
14. Livermore DM. 1995. beta-Lactamases in laboratory and clinical resistance. Clin Microbiol Rev. 8:557-584.
15. Jacoby GA.2009. AmpC beta-lactamases. Clin Microbiol Rev.22:161-182.
16. Gupta K, Hooton TM, Naber KG, et al. 2011. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis.52:e103-120.

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