Challenge M234-3

February 2024

Sputum: *Serratia marcescens* – carbapenem resistant

HISTORY

A simulated sputum sample collected from a 59 year old in-patient with hospital acquired pneumonia was sent to category A laboratories. Although a Gram slide was not provided, laboratories were instructed to consider the sample suitable for culture.

Participants were expected to isolate and report Serratia marcescens and report susceptibility results.

CMPT QA/QC/STATISTICS

All simulated sputum samples are produced at CMPT according to CMPT internal protocols. The sample contained a pure culture of *Serratia marcescens.*

The susceptibility profile of the organism was

Ampicillin: R (>16)
Amikacin: S (≤4)
Amoxicillin-clavulanate: R (16/8)
Aztreonam: R (>8)
Imipenem: R (>8)
Meropenem: R (>16)
Ertapenem: R (>1)
Ciprofloxacin: S (≤0.125)
Cefazolin: R (>16)
Cefoxitin: R (>16)
Ceftriaxone: S (1)
Ceftazidime: S (≤0.5)
Gentamicin: S (≤2)
Levofloxacin: S (≤0.5)
Trimethoprim-sulfamethoxazole (SXT): S (≤0.5/9.5)
Tigecycline: S (2)
Tobramycin: S (≤2)
Piperacillin-tazobactam: S (≤4/4)

The samples are assessed for homogeneity and stability using in-house quality control methods and random selection of samples before and during production, and post sample delivery. The number of random samples selected is 15% of the total production batch.

The challenge sample lot was confirmed to be homogeneous and stable for 17 days. Organism identification was confirmed by a reference laboratory.

MAIN EDUCATIONAL POINTS from M234-3

1. Carbapenemase producing organisms are becoming more frequent and may be isolated from almost any specimen type.
2. Despite resistance to carbapenems these isolates may be susceptible to other antimicrobial classes.
3. As carbapenems are often used as antimicrobials "of last resort" for ill patients, it is important to identify and report potential carbapenemase producers rapidly.
4. Early identification of carbapenemase producers is important for Infection Prevention and Control to limit spread of the organisms.
5. Standardization of terms for these carbapenemase producers has not been achieved. "CRE", "CRO", "CPO", and "CPE" were all used to report in this challenge.

All challenge components have in-house assigned values based on the most clinically appropriate result; the most clinically appropriate result is determined by expert committee evaluation. No further statistical analysis is performed on the results beyond that described under "Suitability for grading."

SURVEY RESULTS

Reference laboratories

Identification: 13/13 (100%) labs reported *Serratia marcescens* (4 labs reported CRE, 4 reported CPO)

Susceptibilities: 9/13 labs reported Ampicillin R, 2 labs reported amoxicillin-clavulanate R, 1 lab referred, 1 lab did not report; 10/13 labs reported cefazolin R, 1 lab referred, 2 labs did not report; 5/13 labs reported ceftriaxone S, 4 labs reported it R, 4 labs referred; 11/13 labs reported ciprofloxacin/levofloxacin S, 2 labs did not report; 13/13 labs reported SXT S; 12/13 labs reported carbapenem R, 1 lab did not report.

Participants

Identification: 51/51 (100%) processing laboratories reported Serratia marcescens (Table 1)

Grading

Maximum grade: 28

Reporting *Serratia marcescens* was graded 4.

Reporting the following susceptibility results was graded 4 for each antimicrobial agent:

Ampicillin R, cefazolin R, ciprofloxacin S, gentamicin/tobramycin S, SXT S, and carbapenems R.

Table 1. Identification results

Reported	Total	Grade
Serratia marcescens ± refer	31	4
Serratia marcescens, possible CRE, refer	4	4
Serratia marcescens, possible presence of CPE/CPO, refer	10	4
Serratia marcescens, possible CPO/présence de carbapénémase	4	4
Serratia marcescens, CRE/CRO	2	4
refer	1	ungraded
sample not normally processed	2	ungraded
Total	**54**	

Susceptibility: 39/51 (76%) processing labs reported ampicillin or amoxicillin-clavulanic acid resistant. 35/51 (69%) reported cefazolin resistant; 46/51 (90%) reported ciprofloxacin susceptible; 49/51 (96%) reported gentamicin/tobramycin susceptible; 49/51 (96%) reported SXT susceptible; 42/51 (82%) reported meropenem resistant. There was no consensus for ceftriaxone (18 reported S and 9 reported R) (Table 2A-G)

Suitability for Grading

A challenge is considered suitable for grading if agreement is reached by 80 percent of selected reference group and at least 50 percent of the participants.

Organism identification and susceptibility to ampicillin, cefazolin, ciprofloxacin, SXT, and carbapenems were correctly performed by at least 80 percent of reference laboratories and greater than 50 percent of all laboratories and were thus, determined to be suitable for grading. Susceptibility results for ceftriaxone, did not reach consensus therefore, were not suitable for grading.

Table 2. Antimicrobial susceptibility results

2A - Ampicillin	Total	Grade
R	33	4
Amoxicillin-clavulanate R	5	4
Ampicillin-sulbactam R	1	4
no report	3	0
refer, sample not normally processed	12	ungraded
Total	**54**	
2B - Cefazolin	**Total**	**Grade**
R	35	4
no report	5	0
refer, sample not normally processed	14	ungraded
Total	**54**	

Table 2. Antimicrobial susceptibility results cont'd

2C - Ceftriaxone	Total	Grade
S	18	ungraded
R	9	ungraded
no report	6	ungraded
refer, sample not normally processed	21	ungraded
Total	**54**	
2D - Ciprofloxacin	**Total**	**Grade**
S	46	4
Levofloxacin S	1	4
no report	3	0
refer, sample not normally processed	4	ungraded
Total	**54**	
2E - Gentamicin/Tobramycin	**Total**	**Grade**
S	49	4
no report	1	0
refer, sample not normally processed	4	ungraded
Total	**54**	
2F - SXT	**Total**	**Grade**
S	49	4
no report	2	0
refer, sample not normally processed	3	ungraded
Total	**54**	
2G – Carbapenems	**Total**	**Grade**
R	42	4
no report	6	0
refer, sample not normally processed	6	ungraded
Total	**54**	

Table 3. Carbapenemase detecting methods

	Result		
Method	**Positive**	**Negative**	**Total**
NG Carba 5	2 (NDM)	13	15
mCIM	6		5
Carba NP	1		1
PCR		1	1
Total	**9**	**14**	**23**

mCIM: Modified carbapenem inactivation test

COMMENTS ON RESULTS

Almost all laboratories identified the isolate correctly. Many laboratories indicated that the isolate was a potential carbapenemase producer and while this aspect was not graded, it provided useful information for Infection Prevention and Control.

Most laboratories indicated the isolate was resistant to ampicillin, ampicillin combined with beta lactamase inhibitors, and cefazolin, and were graded 4. A few laboratories did not report these agents and were graded 0. Most laboratories determined the isolate was susceptible to fluoroquinolones, gentamicin and tobramycin, and trimethoprim sulfamethoxazole and were graded 4. A small number of laboratories did not report these agents and were graded 0 as these agents might be useful in treatment, or referred for testing and were ungraded. The laboratories that reported carbapenems, reported them as resistant and were graded 4. Ceftriaxone results could not be graded for lack of consensus.

ISOLATION AND IDENTIFICATION

The genus *Serratia* is now a member of the family *Yersiniaceae* within the order *Enterobacterales*. [1] *Serratia* grow on a variety of commonly used agar media (e.g., blood agar, MacConkey agar) within 16-18 hours. *Serratia*'s colony morphology is similar to other members of the *Enterobacterales* (circular; grey) and may be mucoid in some instances. Isolates of *Serratia marcescens* may have a red/pink pigment (due to prodigiosin production) but most commonly are unpigmented.

Members of the genus *Serratia* are easily identified by automated biochemical identification systems, MALDI-TOF, and other commercial methods. *Serratia* is unique among *Enterobacterales* in that it produces DNase, lipase, and gelatinase. *Serratia marcescens* is the most common species of *Serratia* isolated from human infections. [2]

ANTIMICROBIAL SUSCEPTIBILITY

Serratia marcescens is intrinsically resistant to ampicillin/amoxicillin/amoxicillin-clavulanate, first-and second-generation cephalosporins (including cephamycins), nitrofurantoin, and colistin.[3] AST results for *Serratia marcescens* should be reported as resistant to each of the listed antimicrobial agents (if they are appropriate to report) regardless of how they test *in vitro*.

CNS infections caused by species of *Enterobacterales* with the potential to express inducible or mutation-based de-repressible ampC enzymes on therapy (e.g., *Serratia marcescens*) [4,5] should not be treated with third-generation cephalosporins such as ceftriaxone and cefotaxime.[2] In the absence of β-lactams, only trace amounts of ampC are produced. [4,5] β-lactams possess different abilities to induce expression of ampC and select for sub-populations with mutations in their promoter sequence regions of ampC that lead to stable over-expression (de-repression) of ampC. Second- and third-generation cephalosporins are good mutant selectors for de-repression while penicillins, carbapenems, and cephamycins are poor selectors. In contrast, third-generation cephalosporins and tazobactam are poor selectors of ampC induction while imipenem, cephamycins, and ampicillin are good inducers of ampC expression. [4,5]

In instances where it is appropriate to report third-generation cephalosporins for an isolate of *Enterobacterales* known to carry chromosomal ampC genes and the isolate initially tests as susceptible to these agents, laboratories may recommend testing subsequent isolates from infected patients treated with third-generation cephalosporins at intervals of 3-4 days if clinically indicated. [3] Derepression is seen most commonly in *Citrobacter freundii* complex, *Enterobacter cloacae* complex *and Klebsiella* [formerly *Enterobacter*] *aerogenes.*

Occasionally, carbapenem-resistant isolates of *Serratia marcescens* have been isolated in Canada; these isolates have been reported to carry the class A SME-type carbapenemase gene.[6]

The *Serratia marcescens* enzymes (SMEs) are Ambler Class A (serine, non metalloenzyme) carbapenem-hydrolysing β-lactamases.[7] They distinctively express resistance to carbapenems and aztreonam, while remaining susceptible to extended-spectrum cephalosporins. [8]

Despite showing susceptibility to third generation cephalosporins, these organisms pose a therapeutic challenge since, as mentioned earlier, de-repressed ampC expressor strains can be selected during treatment leading to clinical failure. [9] This may lead to few therapeutic options for these organisms.

Recent studies have demonstrated the activity of meropenem-vaborbactam combination against SME producers [10] rising as a potential option for severe SME-producing infections

It is also important to mention that although the prevalence of SME-producing *S. marcescens* currently appears low, it is likely underestimated because none of the commercially available molecular carbapenemase detection methods include bla_{SME}.[11]

Carbapenemase detection methods

Decreased susceptibility to any of the carbapenems, ertapenem, imipenem, meropenem and doripenem may result in further testing for carbapenemases. Slight elevations in the MIC of ertapenem can be a result of porin down regulation, especially in the presence of ampC or ESBL enzymes that have low intrinsic carbapenemase activity.

The identification of specific carbapenemases may be important for treatment, as metallo-beta-lactamases are more challenging to treat, and for epidemiological purposes.

CarbaNP

This test is described in the CLSI document M100. The test uses isolated bacterial colonies and is based on in vitro hydrolysis of a carbapenem, imipenem and production of a carboxylic derivative which decreased the pH of the medium, detected by a change in color of a pH indicator.[12] It is a rapid test, but may be less able to detect some perhaps less active types of carbapenemase (like the OXA enzymes).

Modified carbapenem inactivation test (mCIM) and EDTA CIM (eCIM)

This test is described in the CLSI document M100. The test involves incubating the organism in a broth in the presence of a carbapenem containing disk. If the organism produces a carbapenemase, the drug in the disk will be inactivated and won't be able to inhibit a lawn of susceptible *E. coli*. The addition of EDTA allows for the identification of metallo-beta-lactamase (MBL) enzymes as well.[14] This test has the advantage that no special reagents are needed, but it requires overnight incubation.

NG Carba 5

This is a qualitative lateral flow immunoassay for the detection and differentiation the five most prevalent carbapenemases families (NDM, IMP, VIM, OXA-48 and KPC). [15]

KPC plus MBL confirmation ID kit (Rosco Diagnostica, Denmark)

The test uses disk diffusion and compares zones of inhibition of carbapenem alone and in the presence of specific inhibitors.[13]

CLINICAL RELEVANCE

S. marcescens is the most commonly isolated *Serratia* species in human infections and has been recovered from a large variety of clinical specimens.[16] *S. marcescens* has been associated with meningitis, urinary tract infections, pneumonia, bloodstream infections, and wound infections.[16]

CPE or carbapenamase producing *Enterobacterales* are of concern due to their ability to cause health care associated outbreaks and transfer resistance as most enzymes are plasmid encoded.

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