

Challenge M093-4

November 2009

Knee aspirate: *Corynebacterium jeikeium* (companion to G093)

HISTORY

The sample was sent to category A laboratories as a gram smear from a knee aspirate of a 27 year old elite athlete with a prosthetic knee implant showing signs of infection.

CMPT QA

The sample yielded 1+ pure, tiny, white colonies on blood agar plates. The culture was viable for 17 days and was identified as *Corynebacterium jeikeium*.

CLSI indicates that antibiotic susceptibility testing may be warranted for isolates from normally sterile sites, especially in immunodeficient patients. If testing is done, CLSI recommends primary testing for penicillin, vancomycin, erythromycin and gentamicin. Other antibiotics that may be considered include imipenem/meropenem, cefotaxime/ceftriaxone, ciprofloxacin, and clindamycin.

SURVEY RESULTS

Overall, 66% of the participant laboratories reported the isolate as *Corynebacterium jeikeium* or *Corynebacterium* species and received a grade of 4.

Eleven percent of the laboratories identified the isolate as *Kocuria rosea* or *Kocuria* species, and received a grade of zero. [Note: *Kocuria* genre comprises the species formerly known as *Micrococcus roseus*, *Micrococcus varians*, and *Micrococcus kristinae*. They are gram positive cocci occurring mostly in clusters and tetrads. They are catalase positive and non motile.]

Five participants identified the isolate as coagulase-negative staphylococci (CNS) and were graded zero. The laboratories reporting gram positive bacilli, resembling coryneforms were given a grade of 4, if they indicated they would refer the sample.

Note: 1 mark was deducted from the culture report grade if the organism was reported as a "diphtheroid sent to reference lab". The deduction was necessary because the isolate was from a sterile site and a gram positive rod that is grown in culture should be identified to the genus level. The term "diphtheroid" may be suggestive of a contaminant.

Those laboratories reporting gram positive cocci were given a grade of zero.

API Coryne was the best method for the identification of the isolate. Those using classical methods was only able to identify the isolate to

Grading

Maximum grade: 12

A grade of 4 was given to those laboratories that correctly identified the microorganism as *Corynebacterium jeikeium*, *Corynebacterium* species, or described the microorganism as coryneform and mentioned that they would refer the sample for further testing.

Those laboratories that reported gram positive bacilli/coccobacilli were downgraded to 1. Participants that reported gram positive cocci were graded zero.

A grade of 4 was awarded to the participants per antimicrobial agent tested [vancomycin (S) and or penicillin (R)]. Erythromycin, clindamycin, ciprofloxacin and carbapenems were not graded.

Not reporting susceptibility was graded as zero.

Table -1: Reported results for M093-4 -Identification-

| Reported results | Total | % | Grade |
|---|-----------|------------|----------|
| <i>Corynebacterium jeikeium</i> , +/- presumptive, snnp, refer | 44 | 58 | 4 |
| <i>Corynebacterium</i> sp., cannot rule out <i>C.jeikeium</i> , refer | 6 | 8 | 4 |
| gram positive bacilli, resembling suggestive of coryneforms, -refer | 2 | 3 | 4 |
| Diphtheroid +/- refer; gram positive bacilli, suggestive of diphtheroids, refer | 3 | 4 | 3 |
| CNS, +/- snnp, refer | 5 | 7 | 0 |
| <i>Rothia</i> sp.? cocci or diphtheroids, difficult to determine | 1 | 1 | 0 |
| aerobic gram positive bacilli, +/- coccobacilli, refer | 4 | 5 | 1 |
| gram positive cocci, refer | 2 | 3 | 0 |
| <i>Kocuria rosea</i> / <i>Kocuria</i> species, +/- presumptive, refer | 8 | 11 | 0 |
| snp | 1 | 1 | ungraded |
| Total | 76 | 100 | |

Snp: sample not normally processed

the genus *Corynebacterium*.

All participants using RapID CB Plus system correctly identified the isolate as *Corynebacterium jeikeium*. Vitek2 was not able to properly identify the isolate in more than 50% of the cases (11 out of 20).

G093- M093-4 correlation

Fourteen laboratories identified the isolate as gram positive cocci (CNS, *Kocuria* or gram positive cocci). Of those laboratories, only 2 had reported coccobacilli in the gram stain (G093), while the rest reported gram positive cocci. Although incorrect, most of the identification challenge reports matched the morphology observed in the gram challenge.

Table -2: Reported results for M093-4 – Susceptibility testing methods used-

| Methods | No of labs | % |
|------------------|------------|------------|
| Etest | 15 | 30 |
| Kirby-Bauer (KB) | 10 | 20 |
| KB, Etest | 14 | 28 |
| refer | 11 | 22 |
| Total | 50 | 100 |

Susceptibility testing (see Tables 2 and 3)

Susceptibility testing was evaluated only for those participants that identified the strain at least to the genus level (50 laboratories). No scoring for susceptibility testing was done for sites that identified the strain as *Kocuria species* or a coagulase negative staphylococcus as the CLSI interpretation criteria are different from those used for *Corynebacterium*.

Overall, 72% of the laboratories that identified the strain as *Corynebacterium* performed susceptibility testing using either Etest (30%), Kirby-Bauer (20%), or a combination of Etest and Kirby-Bauer (28%).

The Kirby-Bauer (KB) method is not standardized and CLSI guidelines do not give break-

points for disk diffusion. CLSI recommends using broth MIC methods and indicates that often blood supplemented media is required to ensure adequate growth for susceptibility testing. Although not mentioned by CLSI, it is generally accepted that E-test can be used to provide MIC results in place of broth dilution methods. As such, those labs that used KB testing should review their protocols so that MIC based methods are used or the isolates are sent to a reference lab to prevent the reporting of unreliable results.

Reference laboratories:

Penicillin: 13/15 labs reported resistant, one lab did not report, 1 lab indicated susceptibility testing not standardized – GRADED

Erythromycin: 6 labs reported resistant, 1 lab reported susceptible, 2 labs referred, 1 lab indicated susceptibility testing not standardized, 5 labs did not report – UNGRADED

Vancomycin: 13/15 labs reported susceptible, one lab referred, one lab indicated susceptibility testing not standardized - GRADED

Clindamycin: 11/15 labs reported resistant, 1 lab referred, 1 lab indicated susceptibility testing not standardized, 1 lab did not report – UNGRADED

Ciprofloxacin: 7 labs reported resistant, 6 labs did not report, 1 lab referred, 1 lab indicated susceptibility testing not standardized – UNGRADED

Imipenem/Meropenem: 7 labs reported resistant, 6 labs did not report, 1 lab referred, 1 lab indicated susceptibility testing not standardized – UNGRADED

Gentamicin: only 5 out of 15 reference laboratories reported results. All of them reported the strain as Resistant - UNGRADED

The laboratories that performed susceptibility testing reported that the isolate was resistant to all antibiotics tested except vancomycin.

AST: Kirby-Bauer (disk diffusion) and Etest

The KB method is not standardized and CLSI guidelines do not give breakpoints for disk diffusion. CLSI recommends using broth MIC methods and indicates that often blood supplemented media is required to ensure adequate growth for susceptibility testing.

Although not mentioned by CLSI, it is generally accepted that E-test can be used to provide MIC results in place of broth dilution methods. As such, those labs that used KB testing should review their protocols so that MIC based methods are used or the isolates are sent to a reference lab to prevent the reporting of unreliable results.

The Committee recommends that all Proficiency Testing samples should be processed as routine samples even when there is a staff shortage or high workload.

Table -3: Reported results for M093-4 –Susceptibility results-

| Reported | Penicillin | Erythromycin | Vancomycin | Gentamicin | Clindamycin | Ciprofloxacin | Carbapenems |
|----------|------------|--------------|------------|------------|-------------|---------------|-------------|
| R | 37 | 12 | | 11 | 32 | 22 | 14 |
| I | | 1 | | | | | |
| S | | 2 | 35 | | | | |
| NR | | 15 | 2 | | 5 | 12 | 18 |
| N/S | 1 | 1 | 1 | | 1 | 1 | 1 |
| refer | 12 | 19 | 12 | | 12 | 15 | 17 |

NR: not reported; N/S: not standardized

Some laboratories reported the isolate as intermediate (1) or resistant (2) to erythromycin.

All these antibiotics have standardized interpretation criteria according to CLSI document M45-A. Those laboratories that reported “not standardized” to some or all antibiotics should review their protocols and correlate them with M45-A.

ISOLATION and IDENTIFICATION

Corynebacteria are always catalase positive, and the medically relevant species are all non-motile. The genus *Corynebacterium* includes both fermenting and nonfermenting species ¹.

Corynebacterium group JK was first recognized as a distinct species in 1976 ² and was later designated as *C. jeikeium* in 1987. *C. jeikeium* is a pleomorphic gram-positive rod, which varies in form from coccobacillary to bacillary, to club shaped. It is nonhemolytic on standard media, and forms small gray-white colonies on blood agar plates incubated under CO₂ ^{2, 3, 4}.

Biochemically, the organism is relatively nonreactive. It is oxidase-negative, urease-negative, and nonmotile. It does not grow on MacConkey' agar and does not reduce nitrate ². These non-pigmented bacteria do not alkalize citrate, peptonize litmus milk, or produce a halo on Tinsdale medium. *C. jeikeium* is a lipophilic organism and produces turbid growth in brain-heart infusion broth supplemented with 1% Tween 80 ³.

The RapID CB Plus system correctly identifies *C. jeikeium*, as does the API Coryne system, if ancillary tests such as motility, CAMP reaction, or lipophilia are used ^{5, 6}.

ANTIMICROBIAL SUSCEPTIBILITY

According to CLSI adequate studies have not been conducted to recommend reproducible disk diffusion breakpoints, thus only the broth dilution test should be performed when testing antimicrobial susceptibility for *Corynebacterium* species

The medium used for broth dilution should be cation-adjusted Mueller-Hinton broth with lysed horse blood (2.5 to 5% v/v). If testing daptomycin, the medium should contain 50ug/mL calcium.

CLSI recommends that penicillin, vancomycin, erythromycin, and gentamicin be used for primary testing and reporting.

Some species of *Corynebacterium* may exhibit resistance to multiple drug classes. Among the *Corynebacterium* species, *C. jeikeium* and *C. urealyticum* are often multidrug resistant, including resistance to β -lactams, macrolides, and aminoglycosides.

According to CLSI guidelines, resistant results can be reported at 24 hours, but isolates demonstrating susceptible results for β -lactams should be re-incubated and results reported at 48 hours ⁷.

The microorganism is typically susceptible to vancomycin and resistant to penicillin. It has demonstrated variable susceptibility to erythromycin, tetracycline, rifampin, and quinolones. Telithromycin, linezolid and quinupristin/dalfopristin have shown good in vitro activity against *C. jeikeium* ⁸.

Since *C. jeikeium* infections are mostly related to nosocomial sepsis, for which vancomycin has been the mainstay of therapy, there is very limited clinical experience with the use of oral agents that demonstrate in vitro activity, like rifampin, quinolones, tetracyclines and linezolid, for the treatment of these infections ⁹.

CLINICAL RELEVANCE

Corynebacterium jeikeium is a normal commensal organism in healthy adults. It is a lipophilic organism, which explains its predilection for the axilla and groin (both sites of sebaceous glands). *C. jeikeium* is one of the most frequently detected corynebacteria in clinical specimens. *C. jeikeium* most commonly causes sepsis, followed by skin lesions and nodular pulmonary infiltrates, but cases of endocarditis, osteomyelitis, arthritis, visceral abscesses, meningitis, otitis, and other infections have been described. Mortality has been reported to be as high as 34% ⁶. It is becoming well characterized as a pathogen in neutropenic hosts with indwelling catheters, by far the two strongest risk factors for infection ^{5, 6}.

Prosthetic joint infections (PJI) occur in approximately 1.5% to 2.5% of all primary hip or knee arthroplasties ¹¹. Treatment of PJI usually requires either removal of the prosthesis followed by a disabling arthrodesis, or resection of the bioprosthetic materials, stabilization of the joint and administration of 6 weeks of intravenous antibiotics followed by implantation of a new joint prosthesis. Few cases of PJI by *C. jeikeium* have been reported to date ^{12, 13}.

Clinical significance of coryneforms

Estimating the clinical significance of coryneform bacteria isolated from clinical specimens is often difficult for clinical microbiologists. Current recommendations suggest that coryneform bacteria should be identified to the species level when; (i) detected from normally sterile body sites, (ii) from adequately collected clinical material where they are the predominant organism and (iii) from urine specimens where they are the only organism at > 10⁷ cfu/L or are the predominant organism at > 10⁸ cfu/L.¹

The clinical significance of coryneform bacteria is strengthened when multiple specimens are positive for the same coryneform bacteria; and/or coryneform bacteria are seen in the direct Gram stain with a strong presence of leukocytes is also observed ¹.

Identifiers

CMPT has observed that a common error by participant laboratories is to mix samples and results

Participants are encouraged to incorporate two CMPT identifiers on the results report form, which would prevent these type of errors.

TREATMENT

Treatment for *C. jeikeium* is limited by antimicrobial resistance. All early investigators noted pronounced drug resistance, with many isolates susceptible only to vancomycin. *C. jeikeium* is now known to be commonly resistant to β -lactams, aminoglycosides, macrolides, and tetracycline¹⁴. The current standard of care is vancomycin. To date, there have been no reports of vancomycin resistance in *C. jeikeium* isolates. Sanchez Hernandez et al.⁸ found good activity with teicoplanin, telithromycin, linezolid, and quinupristin-dalfopristin indicating possible treatment strategies for highly multidrug-resistant *C. jeikeium*¹⁰.

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