Emergence of coryneforms in osteomyelitis and orthopaedic surgical site infections

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RESEARCH

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Abstract

Background

Coryneform species other than *Corynebacterium diphtheriae* are coming up as important pathogens with the potential to cause serious and life-threatening infections not only in immunocompromised but in immunocompetent individuals as well. The exact infectious potential of these bacteria and their rational antimicrobial treatment is a challenging but essential task.

Method

The study was conducted in the Department of Microbiology and the Department of Orthopaedics, JNMCH, AMU, Aligarh between August 2007 and May 2009. Pus samples were collected from patients of osteomyelitis and other bone infections including orthopaedic surgical site infections. The *Corynebacterium* species isolated in the study was identified using standard microbiological techniques and antimicrobial sensitivity testing was done by Kirby bauer disc diffusion method.

Results

A total of 312 *Corynebacterium* species were isolated. The majority of the coryneforms were isolated from the immunocompetent patients 270 (86.54%). *C.jeikium* was the most common coryneform isolated. Nearly half of the patients 153 (49.04%) had acute infection caused by *Corynebacterium* species after orthopaedic surgery, a quarter 66 (21.15%) had chronic infection and 72 (23.08%) patients had device-related infection. Coryneforms exhibited maximum resistance to aminoglycosides (58.65%) and β-lactams (penicillin group- 57.55%. *C.jeikium* was found to be the most resistant amongst all the *Corynebacterium* species.

Conclusion

The study highlights the fact that the coryneforms are no longer just opportunistic pathogens but they are also becoming important pathogens among immunocompetent individuals as well. The emergence of drug resistance amongst these isolates is of most concern. More studies should be done on identification and on antimicrobial susceptibility of these organisms for the proper treatment of patients with such infections.

Key Words

Coryneforms, identification, infection, resistance, orthopaedics.

What this study adds:

1. Coryneforms are upcoming pathogens with very few studies done all over the world.
2. This is probably the first study on coryneforms from northern India.
3. More studies should be done on the emergence of drug resistance in coryneforms.

Background

A large number of *Corynebacterium* species other than *C.diphtheriae* have been found as part of indigenous human flora and are capable of causing disease.[1] Several species such as *C.xerosis, C. amycolatum, C.striatum, C.minutissimum, C.pseudodiphtheriticum, C. matruchotii, C.aquaticum, C.genitalium* and *C. pseudogenitalium* have been related to human infections. *C.jeikeium* and *C. urealyticum* are well established human pathogens exhibiting resistance to several antibiotics.[2,3]

These organisms are reported to cause serious infections such as bacteremia, valvular endocarditis, neurosurgical shunt infections, meningitis, brain abscess, peritonitis, osteomyelitis, septic arthritis, pneumonia, empyema and urinary tract infections.[4] *Coryneform* bacilli have been found in upto 10% cases of bone and joint infections[5,6] and they have been increasingly isolated (upto 60%) as nosocomial pathogens.[7,8] There have been reports of rare *Coryneform*

species causing infections of bone joints as well as prosthetic joint infections.[9]

The exact infectious potential of these bacteria and their rational antimicrobial treatment can only be established by careful species determination and susceptibility examination. Phenotypic identification along with determination of clinical significance of these species is difficult but necessary and inevitable. In 1981, Hollis and Weaver were the first to systematically examine the full range of coryneform bacteria isolated from clinical specimens.[9] However, recent identification of several new taxa and increased diversity of *Coryneform* bacterial strains encountered in clinical specimens render phenotypic identification even more challenging.[10,11]

The aim of this study was identification and antimicrobial sensitivity testing of clinically relevant *Corynebacterium* species in patients of osteomyelitis and from orthopaedic surgical site infections.

Method

The study was approved by the institute ethical committee, J.N. Medical College, Aligarh Muslim University, Aligarh,

A total of 4,885 pus samples from patients of osteomyelitis and other bone infections including the orthopaedic surgical site infections were screened for coryneforms from indoor patients of the Orthopaedics Unit of Jawaharlal Nehru Medical College Hospital, Aligarh, India during a period from August 2007 to May 2009 in the Department of Microbiology.

All the patients in the study were divided into two groups: 1) Immunocompromised: patients were considered to be immunocompromised when they had history of HIV, malignancy, diabetes mellitus or any other chronic disease. Patients without any such history, but with an age greater than 60 years or a differential cell count showing leucopenia were considered to be immunocompromised. A total of 576 patients were enrolled in this group. 2) Immunocompetent: patients with no underlying chronic disease and not giving any of the above mentioned history were considered to be immunocompetent. A total of 4,309 patients were included in this group.

On the basis of type of infection, the subjects were classified into four groups: 1) patients with acute surgical site infections (SSI); 2) patients with chronic SSI; 3) patients with osteomyelitis; and 4) patients with device-related infections. SSIs were defined as those occurring within 30 days after the operative procedure if no implant is left in place or within one year if an implant was placed and the infection appears to be related to the operative procedure with the patient having either of the following features: purulent discharge, or an organism isolated from an aseptically obtained culture, or signs and symptoms of infection like pain, tenderness, localised swelling, redness or heat or the incision spontaneously dehisces.[12] SSI were defined as chronic, when they did not appeared to follow the normal healing process in less than four weeks. Acute SSI were defined as those that followed the normal phases of healing; and showed signs of healing in less than four weeks.[13,14] Patients were considered to have osteomyelitis when the organism was cultured from bone or there was evidence of osteomyelitis on direct examination during a surgical operation or at least two of the following signs/symptoms were present with no other recognised cause: fever (38˚C), localised swelling, tenderness, heat or drainage at suspected site of bone infection.[12] Device-related infections were defined as those occurring 72 hours after insertion of the medical device.

Direct microscopic examination was done by Gram staining, followed by culture on 5% sheep blood agar, MacConkey agar and enrichment was done in brain heart infusion broth with 1% serum. Incubation was done for 18–24 hours at 37˚C.

Since *coryneforms* could be confounders as they are a part of indigenous human flora, isolated *Corynebacteria* species were considered pathogenic when at least one of the following criteria were met: 1) clinical history of inflammation, tenderness, purulent discharge with or without fever; 2) in cases of superficial samples, isolation at least three times in samples taken at three different times and the presence of polymorphonuclear neutrophils on Gram's staining; 3) isolation from a deep sample; 4) in any kind of sample, the presence of gram positive bacilli within polymorphonuclear neutrophils on gram's staining.[15]

Identification of *Coryneform* species was done using standard biochemical methods.[16] All the isolated *Coryneform* species were subjected to antimicrobial susceptibility testing using Kirby bauer disc diffusion method on Mueller Hinton agar incorporated with 5% sheep blood.[17] The antimicrobial agents used were ampicillin 10µg, oxacillin 1µg, cefazolin 30µg, cefotaxime 30µg, cefoperazone-sulbactum 75/10µg, erythromycin 15µg, gentamicin 10µg, amikacin 30µg, chloramphenicol 30µg, tetracycline 30µg, ofloxacin 5µg, gatifloxacin 5µg, vancomycin 30µg, teicoplanin 30µg, and linezolid 30µg.

Results

A total of 312 *Corynebacterium* species were isolated from pus samples of the indoor and the patients of the Orthopaedics Unit of the Jawaharlal Nehru Medical College

and Hospital. *Coryneforms* were isolated in 6.3% of the immunocompetent individuals and in 7.3% of the immunocompromised individuals. *Coryneforms* from the immunocompetent patients accounted for 270 (86.54%) isolates, whereas 42 (13.46%) isolates were from patients with an immunocompromised status. The average age of the immunocompetent individuals was 32.04 years ± 0.44, while the average age of the immunocompromised individuals was 67 years ± 0.24 (p value <0.001). Amongst the immunocompromised patients in the study 33 (78.57%) had malignancy and 9 (21.43%) were diabetic. *C.jeikium* was the most common coryneform isolated both from the immunocompetent as well as immunocompromised group. *C.amycolatum, C.striatum* and *C.minuissimum* followed *C.jeikium* among the immunocompetent patients whereas *C.minutissimum* was the second most common coryneform after *C.jeikium* in the immunocompromised group (Table 1).

Nearly half of the patients 155 (49.67%) had acute infection from *Corynebacterium* species after orthopaedic surgery, a quarter 66 (21.15%) had chronic infection and 70 (22.43%) patients had device-related infection (Table 2).

Microbiology: Corynebacterium jeikium was the commonest coryneform species isolated in all the types of orthopaedic infections, making a total of 155 (49.68%). The majority of the *C.jeikium* isolates 96 (62.74%) were from patients with acute orthopaedic surgery. *C.striatum* 45 (14.42%) was equally isolated from patients with acute (14 isolates) and chronic (13 isolates) orthopaedic surgical infection as well as in device-related infections (14 isolates). However, only four *C.striatum* strains were isolated from patients with osteomyelitis. The other coryneform species isolated in decreasing order of frequency were *C.amycolatum* 39 (12.50%), *C.minnutissimum* 38 (12.18%), *C.ulcerans* 12 (3.85%), *C.urealyticum* 11 (3.52%), *C.macgenlii* 3 (0.96%), *C.xerosis* 2 (0.64%) and *Microbacterium* species 2 (0.64%).

In patients with device-related infections *C.jeikeium* 23 (31.94%) was the commonest *Corynebacterium* species isolated followed by *C.minnutissimum* 16 (22.22%), *C.striatum* 14 (19.44%) and *C.urealyticum* 11 (15.28%).

Antimicrobial susceptibility pattern : *C.jeikium* was found to be the most resistant amongst all the *Corynebacterium* species. Coryneforms exhibited maximum resistance to aminoglycosides (58.65%) and β-lactams (penicillin group 57.55%, cephalosporins 58.76%) followed by macrolides (56.10%) and flouroquinolones (45.00%). Among the β-lactam group of antimicrobials, penicillins (45.5%) were found to have a slightly better spectrum than cephalosporins (cefazolin 43.6%, cefotaxime 39.4%). Among the cephalosporins the susceptibility pattern of cefazolin and cefotaxime was almost the same and more noteworthy was no increment in the sensitivity profile on addition of the β-lactamase inhibitor (cefoperazone-sulbactum 40.71%).

Among the aminoglycosides amikacin 140 (44.9%) was found to have the better spectrum of activity compared to gentamicin 118 (37.8%). For the fluoroquinolones tested (i.e., ofloxacin 57.4% and gatifloxacin 52.6%) the susceptibility pattern was not significantly different.

Chloramphenicol and tetracycline, antimicrobials which are not routinely used nowadays had a good sensitivity profile with sensitivity in between the range of 60%–90% for different *Coryneform* species. The glycopeptides vancomycin and teicoplanin were found to be active against all the *Coryneform* species except *C.jeikeium* where 5 (3.2%) isolates were resistant. Linezolid was the only drug having a uniform sensitivity of 100% for all the *Coryneform* species.

Discussion

After long lasting discussion and confusion about their clinical significance, coryneforms have emerged as important pathogens.[18] The *Corynebacterium* species have been the focus of attention as there are increasing reports of their isolation from patients with various infections, in particular from people with decreased immunity as well as in normal immune conditions. In our study, a total of 312 pus samples from various surgical and non-surgical sites of bone infection showed the growth of coryneforms. The majority of the isolates were from immunocompetent patients (86.54%), although coryneforms are thought to be opportunistic pathogens causing infection in patients with decreased immunity. There was a significant difference in the average age of the two groups (p< 0.001); while in the immunocompetent group it was 32 years, in the immunocompromised it was much higher at 67 years. The fact that the majority of the cases were immunocompetent young individuals suggests unexplored virulence potential of this group of bacteria. However the fewer number of immunocompromised patients usually reporting to the orthopaedics unit of the hospital could be a confounding factor.

Patients with acute infection accounted for nearly 50% of the coryneform isolates in the study. These findings point towards the pathogenic potential of the *Coryneform* species. Patients with chronic infection and device-related infection accounted for nearly a quarter each, of the total coryneforms isolated. *Coryneforms* are a known cause of infection in immunocompromised patients as well as in device-related and hospital acquired infections.[18] It is

considered that chronic infection leads to long-term hospital admission, prolonged antimicrobial therapy, thereby compromising the immune status of the patient further and increasing the risk of hospital acquired infections. [19,20]

In our study *C.jeikium* was the commonest Corynebacterium isolated in all types of orthopaedic infections, followed by *C.striatum.* *C.jeikium* has been reported as an important cause of infection in immunocompetent as well as immunocompromised individuals and in device-related hospital acquired infections.[3,21] It has been reported in infections following prosthetic joints, and open fractures,[6] total knee arthroplasty infection,[22] and osteomyelitis with foreign body.[23] Similarly the presence of *C.striatum* infection following prosthetic joint infection, open fracture and vertebral osteomyelitis has been reported previously.[24]

The other *Coryneform* species isolated in our study were *C.amycolatum, C.minnutissimum, C.ulcerans, C.urealyticum, C.macgenlii, C.xerosis* and *Microbacterium* species. These coryneform species along with *C.jeikium* and *C.striatum* are some of the most frequently isolated coryneform bacteria in clinical microbiology.[3,10] On antimicrobial susceptibility testing *C.jeikium* was found to be the most resistant amongst all the coryneform species. It has been reported to exhibit multi-drug resistance in other studies as well.[25,26] Penicillins had the best spectrum among the β-lactam antimicrobials. Resistance was shown to all the group of antimicrobials including the glycopeptides. There have been reports of vancomycin and teicoplanin resistance among the coryneforms.[27] Linezolid was the only antimicrobial to which all the strains were found to have uniform sensitivity.

Conclusion

Our assessment of corynebacteria isolates highlighted the fact that the coryneforms are no longer just opportunistic pathogens but they are becoming important pathogens among immunocompetent individuals as well. The emergence of drug resistance amongst these isolates is of most concern. More studies should be done on identification and on antimicrobial susceptibility of these organisms with rigorous clinical correlation to understand them better for appropriate and timely treatment of patients with such infections.

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CONFLICTS OF INTEREST

None.

Table 1: Isolation of coryneforms in the two different study groups

Corynebacterium species	Group 1 Immunocompetent No.(%)	Group 2 Immuno-compromised No.(%)	Total No.(%)
C.jeikeium	140(51.85)	15(35.71)	155(12.5)
C.striatum	36(13.33)	9(21.43)	45(14.42)
C.amycolatum	36(13.33)	3(7.14)	39(12.50)
C.minnutissimum	28(10.37)	10(23.81)	38(12.18)
C.ulcerans	11(4.07)	1(2.38)	12(3.85)
C.urealyticum	7(2.59)	4(9.52)	11(3.52)
C.pseudodidhtheriticum	5(1.85)	-	5(1.6)
C.macgenlii	3(1.11)	-	3(0.96)
C.xerosis	2(0.74)	-	2(0.64)
Microbacterium species	2(0.74)	-	2(0.64)
Total	270(86.54)	42(13.46)	312(100)

Table 2: Isolation of coryneforms in different types of infections from orthopaedic units

Corynebacterium species	**Acute surgical site infection No.(%)**	**Chronic surgical site infection No.(%)**	**Osteomyelitis No.(%)**	**Device-related infections No.(%)**	**Total No.(%)**
C.jeikeium	96	29	7	23	155(49.68)
C.striatum	14	13	4	14	45(14.42)
C.amycolatum	22	9	2	6	39(12.50)
C.minnutissimum	9	8	5	16	38(12.18)
C.ulcerans	4	3	2	3	12(3.85)
C.urealyticum	1	1	1	8	11(3.52)
C.pseudodidhtheriticum	3	2	-	-	5(1.6)
C.macgenlii	2	1	-	-	3(0.96)
C.xerosis	2	-	-	-	2(0.64)
Microbacterium species	2	-	-	-	2(0.64)
Total	155(49.67)	66(21.15)	21(6.7)	70(22.43)	312(100)