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## Extended culture methods among orthopaedic surgery patients and recovery of various microorganisms in a tertiary care hospital

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### Abstract

**Introduction:** Extended culture has made a marked tremendous impact on orthopaedic post operative treatment due to prolonged incubation period.

**Aim:** To identify various organisms isolated from different surgical procedures in Orthopaedic department and their rapidity of growth of various microorganisms isolated along with their sensitivity patterns.

**Materials and method:** The prospective study was conducted in the department of Microbiology, KIMS, Secunderabad over a period of November 2015 to October 2016. A total of 104 samples were received from orthopaedic department during different orthopaedic surgical procedures. The received specimens were inoculated onto 5% sheep blood agar, chocolate agar, MacConkey agar and fluid thioglycolate medium and incubated at 37°C, 5% CO<sub>2</sub> for 40-48 hours and read daily for the growth. If no growth was observed, subcultures were made from fluid thioglycolate and observed daily till 7 days. Positive growths processed in Vitek 2 compact system (Biomérieux). The data collected was analyzed using Microsoft Excel. UK Standard for microbiology Investigations Investigation for orthopaedic implants associated infections (NICE accredited guidelines 2016) are the basis of the study.

**Results:** A total of 104 patients were investigated in the present study, out of which 60 (57.6%) were culture positive. 44 (42.30%) cultures were sterile after 7 days of incubation. The orthopaedic surgical procedures were 52 Open fractures with Open reduction internal fixation (50%), 10 Septic arthritis (9.61%), 7 Sinovitis (6.7%), 7 Discharging sinus (6.7%) and 29 others (27.8%). Among all culture positives (n=60), 38 Gram negative organisms (61.66%) were in majority as compared to 23 Gram positive microorganisms (38.33%). Among them, 17 E.coli (28.3%), 8 Enterococcus (13.33%), 7 MSSA (6.7%), 5 Pseudomonas (4.8%) were the predominant pathogen.

**Conclusion:** As 15(25%) microorganism showed prolonged incubation (48hrs to 7 days) period, which could be missed in conventional culture method (24-48hrs). The outcome of all culture positive patient were good, 95% successfully treated.

**Keywords:** Extended culture, orthopaedic procedures, microorganisms, culture method

### Introduction

In the present era, infections, are posing a great challenge for treatment options, especially infections of bones and joints due to prolonged incubation period and emerging antibiotic resistance pattern.

The use of implants used in open reduction and internal fixation (ORIF) which are foreign bodies to the body, orthopaedic trauma surgery is at grave risk of microbiological contamination and infection [1, 2, 3].

Gram staining in elective revision cases should not be considered for diagnosing infection as it has extremely poor sensitivity [4, 5, 6, 7, 8]. A negative Gram stain does not however rule out infection. False positive Gram stains associated with periprosthetic infections are rare, but may have severe consequences if used as the basis for treatment<sup>4</sup>. Its sensitivity and specificity of 7-9% and 92-99% respectively [4, 9].

Culture methods should include an enrichment broth. Visual inspection of broth media is done and their time of detection may depend on how frequently cultures are examined [10].

Traditionally orthopaedic samples have been cultured for up to five days.

According to UK Standard for microbiology Investigations (for orthopaedic implants) NICE guidelines, there is no need for incubation times exceeding 5 days [11, 12].

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Timing of administration of prophylactic antimicrobial was considered early operative if it was given more than 2 hours before incision, pre-operative if it was given less than 2 hours before incision, peri-operative if it was during surgery and post-operative if it was given after the completion of surgery<sup>[13]</sup>. However, in cases of suspected prosthetic joint infection, with low virulence organisms, or where preoperative cultures have failed to show growth and the clinical picture is consistent with prosthetic joint infection, culture may be extended to 14 days<sup>[6, 14, 15]</sup>.

**AIM**

**Primary Objectives**

1. To identify various organisms isolated from different surgical procedures in Orthopaedic department, employing extended culture.
2. To know whether a single microorganism is the cause of infection or polymicrobial.
3. To find rapidity of growth of various microorganisms isolated.
4. To identify the types of microorganisms (Gram positive or Gram negative microorganisms).

**Secondary Objectives**

1. To identify the sensitivity patterns according to the types of microorganisms isolated.
2. To identify the predominant age group for culture positive undergoing orthopaedic surgery.
3. To identify gender predominance.

**Materials and method**

**Study Centre**

The study was conducted in the department of Microbiology, Krishna Institute of Medical Sciences Ltd, Secunderabad during a period of November 2015 to October 2016 (1 year). It is a prospective study. During this period 104 samples were received from orthopaedic department with various operative procedures. These procedures were ORIF, Septic arthritis, Discharging sinus, Posttraumatic wound, Infected malunion, Chronic Osteomyelitis, Polytrauma, Cold abscess, Infected THR.

**Patient Selection**

The samples received from different orthopaedic surgical procedure, were collected during the surgical procedure. The samples are collected, taking aseptic precaution and following the standard recommendation. The samples were immediately sent to microbiology laboratory for processing.

**Processing of Specimen**

The sample processing was done in a Biosafety cabinet type IIa using aseptic technique to reduce contamination and to protect the staff.

The received specimens were inoculated onto 5% sheep blood agar, chocolate agar, MacConkey agar and fluid thioglycolate medium. The plates were incubated at 37°C, 10%CO<sub>2</sub> for 40-48 hours. Cultures are read daily for the growth of bacteria. If no growth was observed on the plates, subcultures were made from the fluid thioglycolate broth onto 5% sheep Blood agar, Chocolate agar and MacConkey agar, which were read every day till a period of 14 days. The growth of suspected pathogens were identified by colony morphology, and performing preliminary identification tests (Grams staining, motility, catalase and oxidase). Identification is done by using GN and GP card of Vitek 2 Compact (Biomerieux). Antimicrobial susceptibility testing done by using GN280, GN281, GP628 cards Vitek 2 compact (Biomerieux). Quality Control strains of ATCC recommended for Vitek 2 compact (Biomerieux) were used for above mentioned cards as per manufacturer’s instructions. The data collected was analyzed using Microsoft Excel. UK Standard for microbiology Investigations Investigation for orthopaedic implants associated infections (NICE accredited guidelines 2016) are the basis of the present study.

**Results**

A total of 104 patients were investigated in the present study, out of which 60 (57.6%) were culture positive. 44 (42.30%) cultures were sterile after 7 days of incubation as per the guidelines. The various orthopaedic surgical procedures and recovery of microorganisms in culture is shown in (Table 1). The cultures showing growth of monomicrobial and polymicrobial (2 or 3) is shown in (Table 2). The rapidity of growth of different pathogenic microorganism is shown in (Table 3) and depicting 33 growth of microorganism that could be missed in conventional cuture method. The various types of microorganisms in culture positive growth (Table4). Among all isolates (n=104), 57% (n=60) was culture positive. Among them, Gram positive organism 22.1% (n=23) and Gram negative organism 35.5% (n=37) was observed. The antibiotic sensitivity pattern of Gram negative bacteria is shown in (Table 5a, 5b).

The antibiotic sensitivity pattern of Gram positive bacteria is shown in (Table 5c). The predilection for age was maximum between age of 40-60 yrs (38.16%; n=55). The predominant affected gender was seen in male (77.8%; n=81) than in female (22.1%; n=23).

The below table has compared our study with other studies.

**Table 1:** Various orthopaedic surgical procedures and recovery of microorganisms in culture.

| S. no.      | Micro - organisms           | NBG | E.coli | Enterococcus | MSSA | Pseudomonas | Proteus | MRSA | Morganella | CONS | Klebsiella | Enterobacter | Acinetobacter | Aeromonas | Burkholderia | Granulicatella | Serratia |
|-------------|-----------------------------|-----|--------|--------------|------|-------------|---------|------|------------|------|------------|--------------|---------------|-----------|--------------|----------------|----------|
|             | Name of surgery             |     |        |              |      |             |         |      |            |      |            |              |               |           |              |                |          |
| 1           | ORIF (n=52)                 | 27  | 4      | 3            | 4    | 4           | 1       | 1    | 1          |      | 2          | 1            | 1             |           |              | 1              | 1        |
| 2           | Septic arthritis (n=10)     | 4   | 1      | 3            |      |             | 1       |      |            |      |            |              |               |           | 1            |                |          |
| 3           | Synovitis (n=7)             | 3   | 2      | 1            |      |             |         | 1    |            |      |            |              |               |           |              |                |          |
| 4           | Discharging sinus (n=7)     |     | 2      |              | 1    |             | 1       | 1    |            | 2    |            |              |               |           |              |                |          |
| 5           | Crush injury (n=6)          | 2   | 1      |              |      |             | 1       |      | 1          | 1    |            |              |               |           |              |                |          |
| 6           | Infected THR (n=6)          | 3   |        | 1            |      |             |         |      |            | 2    |            |              |               |           |              |                |          |
| 7           | Cold abscess (n=6)          | 3   | 2      |              |      | 1           |         |      |            |      |            |              |               |           |              |                |          |
| 8           | Polytrauma (n=5)            |     | 3      | 2            |      |             |         |      |            |      |            |              |               |           |              |                |          |
| 9           | Chronic Osteomyelitis (n=4) |     |        |              | 2    |             |         | 1    |            |      |            |              |               | 1         |              |                |          |
| 10          | Lytic lesion (n=3)          |     | 1      | 1            |      |             |         |      | 1          |      |            |              |               |           |              |                |          |
| 12          | Infected non-union (n=1)    |     |        |              |      |             |         |      |            |      |            | 1            |               |           |              |                |          |
| 13          | Post- traumatic wound (n=1) |     | 1      |              |      |             |         |      |            |      |            |              |               |           |              |                |          |
| Total n=104 |                             | 44  | 17     | 8            | 7    | 5           | 4       | 4    | 3          | 3    | 2          | 2            | 1             | 1         | 1            | 1              | 1        |

**Table 2:** Details of culture showing no growth and culture growth positives as monomicrobial and polymicrobial

| S.no. | Culture types                | Microorganisms (Total n=104)   |
|-------|------------------------------|--|
| 1.    | Sterile                      | 44   |
| 2.    | Growth                       | 60   |
| 2a.   | Monomicrobial                | 48   |
| 2b.   | i) with 2 microorganism (3)  | 1. <i>Enterococcus faecium</i> + <i>Morganella morganii</i> 2. <i>Staphylococcus pseudintermedius</i> + <i>Proteus mirabilis</i> 3. <i>E. coli</i> + <i>Enterococcus faecium</i> |
|       | ii) with 3 microorganism (1) | 1. <i>Enterococcus faecium</i> + <i>Staphylococcus cohnii</i> + <i>Staphylococcus aureus</i>   |

**Table 3:** Rapidity of growth by different microorganisms.

| S.no | Microorganisms        | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|------|-----------------------|-------|-------|-------|-------|-------|
| 1    | <i>E. coli</i> (n=17) | 10    | 3     | 3     | 1     |       |
| 2    | Enterococcus (n=8)    | 1     | 3     | 4     |       |       |
| 3    | MSSA (n=7)            | 2     | 3     | 1     |       | 1     |
| 4    | Pseudomonas (n=5)     | 3     | 1     | 1     |       |       |
| 5    | Proteus (n=4)         | 4     |       |       |       |       |
| 6    | MRSA (n=4)            | 1     | 3     |       |       |       |
| 7    | Morganella (n=3)      | 1     |       | 2     |       |       |
| 8    | CONS (n=3)            | 2     |       |       |       | 1     |
| 9    | Klebsiella (n=2)      | 1     | 1     |       |       |       |
| 10   | Enterobacter (n=2)    | 1     | 1     |       |       |       |
| 11   | Acinetobacter (n=1)   | 1     |       |       |       |       |
| 12   | Aeromonas (n=1)       |       |       | 1     |       |       |
| 13   | Burkholderia (n=1)    |       | 1     |       |       |       |
| 14   | Granulicatella (n=1)  |       | 1     |       |       |       |
| 15   | Serratia (n=1)        |       | 1     |       |       |       |
|      | Total (n=60)          | 27    | 18    | 12    | 1     | 2     |
|      |                       |       | 75%   |       | 25%   |       |

**Table 4:** Various types of microorganisms in culture positive growth.

| Total number (n=60) |                      |                       |
|---------------------|----------------------|-----------------------|
| S.no.               | Gram positive (n=23) | Gram negative (n=37)  |
| 1                   | Enterococcus (n=8)   | <i>E. coli</i> (n=17) |
| 2                   | MSSA (n=7)           | Pseudomonas (n=5)     |
| 3                   | MRSA (n=4)           | Proteus (n=4)         |
| 4                   | CONS (n=3)           | Morganella (n=3)      |
| 5                   | Granulicatella (n=1) | Klebsiella (n=2)      |
| 6                   |                      | Enterobacter (n=2)    |
| 7                   |                      | Acinetobacter (n=1)   |
| 8                   |                      | Burkholderia (n=1)    |
| 9                   |                      | Serratia (n=1)        |
| 10                  |                      | Aeromonas(n=1)        |

**Table 5a:** Antibiotic susceptibility pattern of Gram negative microorganism in percentage (%)processed in GN280 card in Vitek Compact2 (Biomérieux)

| S.no. | Microorganism Antibiotics | <i>E. coli</i> (n=17) | Proteus (n=4) | <i>Morganella</i> (n=3) | <i>Klebsiella</i> (n=2) | <i>Enterobacter</i> (n=2) | <i>Serratia</i> (n=1) |
|-------|---------------------------|-----------------------|---------------|-------------------------|-------------------------|---------------------------|-----------------------|
| 1     | Ampicillin                | 0                     | 0             | 0                       | 0                       | 0                         | 0                     |
| 2     | Amoxiclav                 | 0                     | 0             | 0                       | 0                       | 0                         | -                     |
| 3     | Piptaz                    | 0                     | 50            | 0                       | 0                       | 100                       | 0                     |
| 4     | Cefuroxime                | 0                     | 0             | 0                       | 0                       | 0                         |                       |
| 5     | Cef-axetil                | 0                     | 0             | 0                       | 0                       | 50                        | 100                   |
| 6     | Ceftriaxone               | 0                     | 0             | 0                       | 50                      | 50                        | 100                   |
| 7     | Cefperazone-sulbactam     | 0                     | 50            | 0                       | 50                      | 100                       | 100                   |
| 8     | Cefepime                  | 0                     | 50            | 0                       | 50                      | 100                       | -                     |
| 9     | Ertapenem                 | 41.7                  | 50            | 0                       | 50                      | 100                       | 100                   |
| 10    | Imipenem                  | 76.47                 | 75            | 100                     | 100                     | 100                       | 100                   |
| 11    | Meropenem                 | 23.52                 | 75            | 100                     | 100                     | 100                       | 100                   |
| 12    | Amikacin                  | 88.23                 | 50            | 100                     | 100                     | 100                       | 100                   |
| 13    | Gentamycin                | 82.35                 | 50            | 100                     | 100                     | 100                       | 0                     |
| 14    | Ciprofloxacin             | 0                     | 50            | 100                     | 100                     | 100                       | 100                   |
| 15    | Tigecycline               | 100                   | 75            | 100                     | 100                     | 100                       | 100                   |
| 16    | Colistin                  | 100                   | 0             | 100                     | 100                     | 0                         | 100                   |
| 17    | Cotrimoxazole             | 29.41                 | 0             | 100                     | 100                     | 100                       | 100                   |

**Table 5b:** Antibiotic susceptibility pattern of Gram negative microorganism in percentage (%); processed in GN281 card in Vitek Compact 2 (Biomeriux):

| S.no. | Microorganisms  | Pseudomonas (n=5) | Acinetobacter (n=1) | Burkholderia | Aeromonas (n=1) |
|-------|-----------------|-------------------|---------------------|--------------|-----------------|
|       | Antibiotics     |                   |                     | (n=1)        |                 |
| 1     | Tic-clav        |                   | 0                   | 0            | 100             |
| 2     | Piptaz          | 20                | 0                   | -            | 100             |
| 3     | Ceftazidime     | 40                | 0                   | 0            | 100             |
| 4     | Cefepime        | 40                | 0                   | -            | 100             |
| 5     | Doripenem       | 100               | 0                   | -            | 100             |
| 6     | Imipenem        | 60                | 0                   | -            | 100             |
| 7     | Meropenem       | 60                | 0                   | 100          | 100             |
| 8     | Amikacin        | 100               | 0                   | -            | 100             |
| 9     | Gentamycin      | 100               | 0                   | -            | 100             |
| 10    | Ciprofloxacin   | 100               | 0                   | -            | 100             |
| 11    | Levofloxacin    | 100               | 0                   | 100          | 100             |
| 12    | Minocyclin      | -                 | 50                  | 100          | -               |
| 13    | Tigecycline     | -                 | 100                 | -            | -               |
| 14    | Colistin        | 100               | 100                 | -            | 100             |
| 15    | Aztreonam       | -                 | -                   | -            | 100             |
| 16    | Cotrimoxazole   | -                 | -                   | 0            | -               |
| 17    | Chloramphenicol | -                 | -                   | 100          | -               |

**Table 5c:** Antibiotic susceptibility pattern of gram positive microorganism in percentage (%) processed in GP628 card in Vitek Compact2 (Biomeriux)

| S. No. | Microorganisms   | Enterococcus (n=8) | MSSA (n=7) | MRSA (n=4) | CONS (n=3) | Granulicatella (n1) |
|--------|------------------|--------------------|------------|------------|------------|---------------------|
|        | Antibiotics      |                    |            |            |            |                     |
| 1      | Benzylpenicillin | 25                 | 0          | 0          | 0          | 0                   |
| 2      | Oxacillin        | 0                  | 100        | 0          | 0          | 0                   |
| 3      | Gentamycin       | 16.66              | 16.66      | 75         | 66.66      | 100                 |
| 4      | Ciprofloxacin    | 0                  | 100        | 75         | 66.66      | 100                 |
| 5      | Levofloxacin     | 0                  | 66.66      | 75         | 66.66      | 100                 |
| 6      | Clindamycin      | 0                  | 100        | 75         | 33.33      | 100                 |
| 7      | Linezolid        | 100                | 100        | 100        | 66.66      | 100                 |
| 8      | Daptomycin       | 100                | 100        | 100        | 66.66      | 100                 |
| 9      | Teicoplanin      | 87.5               | 100        | 100        | 66.66      | 100                 |
| 10     | Vancomycin       | 62.5               | 100        | 100        | 66.66      | 100                 |
| 11     | Tetracycline     | 0                  | 100        | 100        | 50         | 100                 |
| 12     | Tigecycline      | 85                 | 100        | 100        | 66.66      | 100                 |
| 13     | Cotrimoxazole    | -                  | 50         | 50         | 33.33      | 100                 |

**Conclusion**

As 15(25%) microorganism showed prolonged incubation (48hrs to 7 days) period, which could be missed in conventional culture method(24-48hrs). The outcome of all culture positive patient were good, 95% successfully treated.

**Discussion**

The duration of incubation is not specified in most studies,

but a 5-day period has often been reported<sup>16-18</sup>. Recently, some authors have proposed prolonging the incubation period to 7 or 14 days in order to reveal microorganisms with low virulence, such as *Propionibacterium acnes*, *Peptostreptococcus* spp., and *Corynebacterium* spp.<sup>19-22</sup>. Low-virulence, foreign-material-adherent bacteria are typically in a dormant starved state with a slow replicating-rate<sup>23</sup>.

**Table 6:** Showing Comparison of previous studies with present study.

| S. no. | Study  | No of samples; patients | Incubation period (days) | Early detection of growth (<7days) | Late detection of growth (>7 days) |
|--------|--|-------------------------|--------------------------|------------------------------------|------------------------------------|
| 1      | Schafer <i>et al.</i> (2008) <sup>[20]</sup>   | S: 284; P: 110          | 14                       | 73%                                | 27%                                |
| 2      | Butler-Wu <i>et al.</i> (2011) <sup>[19]</sup> | S: 557; P: 173          | 13                       | 71%                                | 29%                                |
| 3      | Nora Schwotzer <i>et al.</i> (2014)            | S:499 ; P:117           | 14                       | 96.6%                              | -                                  |
| 4      | L. Drago <i>et al.</i> (2015)                  | S:387; P:449            | 15                       | 73%                                | 17%                                |
| 5      | Present study                                  | S: 104; P:78            | 14                       | 100%                               | -                                  |
|        |  |                         |                          | 75% (<48hrs)   25% (>48hrs)        |                                    |

Our study shows that Gram negative infections is most common pathogenic microorganism isolated from 38 cases (63.33%) out of 60 culture positive cases and continue to be a major threat to patients.

However, in clinically suspected cases of fungal and anerobic infections, cultures were done on Sabaraud's dextrose agar and GENbag anaer gaspack (Biomerieux) respectively and were found to be sterile after 7 days.

*E. coli* remains the most common pathogen (28%; n=17 cases), especially in open fractures, Synovitis, Discharging sinus, Polytrauma. *E. coli* is a commensal of gut and as many orthopaedic bedridden patients and contamination of wounds, dressing, linen, clothes and even hands during perineal hygiene plays a major role in increasing chances of transmission of infection.

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