Aerobic and Anaerobic Bacterial Profile of Deep Space Head and Neck Infections in a Tertiary Care Hospital in Kerala

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Abstract
A wide spectrum of aerobic, microaerophilic and anaerobic bacterial pathogens are involved in the causation of deep seated abscesses in the head and neck. Early clinical diagnosis and isolation of the etiologic agent in culture and antibiotic susceptibility testing of the isolate are essential for prompt treatment with appropriate antibiotics, which in turn reduces the morbidity and mortality in these cases. A study was conducted in the Department of Microbiology in association with the Department of Neurosurgery, Government Medical College, Trivandrum and the Department of Oral and Maxillofacial surgery at Government Dental College, Trivandrum, for a period of one year from September 2016 to August 2017, to find out the etiological agents of deep space infections in head and neck both by aerobic and anaerobic culture methods. A total of 95 isolates were obtained from clinical samples collected during that period. The aerobic organisms isolated includes 33.6%, anaerobic organisms 61.05% and microaerophilic isolates 5.2%. The predominant aerobic organism isolated in the study was Streptococcus pyogenes (53.12%). Among the anaerobic organisms Peptostreptococcus species (63.7%) is the most common species isolated. The miccroaerophilic organism isolated in the study was microaerophilic Streptococci (5.2%). Antibiotic sensitivity testing of aerobic and microaerophilic isolates were performed by Kirby-Bauer disc diffusion method and anaerobic isolates by agar dilution method according to CLSI guidelines. The patients were treated with the appropriate antibiotics based on the antibiotic sensitivity pattern of the isolate. The mortality rate in the study was 5%.
Keywords: *Deep space infections, Anaerobes, microaerophilic bacteria.*

Introduction

Deep space infections of head and neck are infections developing within, or spreading to the potential spaces and fascial planes of head and neck, with either abscess formation or cellulitis.[1] Abscesses of the brain arise when the brain is

exposed directly as a result of fracture, infection of an air sinus or at surgery. They also result from haematogenous spread, associated with respiratory and dental infections or endocarditis. In 25 % cases no underlying primary infection is found.[2] In the pre - antibiotic era, deep space infections almost invariably turned out fatal. The incidence of these infections has decreased greatly as a result of modern antibiotics and improvement of oral hygiene and dental care.Now, the incidence of head and neck deep space infections is estimated at around 10 per 100, 000 individuals per year.[3] Clinical manifestations depend on the spaces infected. Common clinical features include pain, fever, swelling, dysphagia, trismus, dysphonia, otalgia and dyspnoea. A rapidly progressive course may be seen in immunocompromised patients. Diagnosis is primarily based on clinical assessment. Imaging techniques help to find out the extent of spread of infection. Mortality rate was high, around 54%, before second world war, now reduced to 4% due to advanced technology, use of appropriate antibiotics and surgical management.[4]

Methodology

Study Design: Descriptive study

Study Setting: Tertiary care hospital : Department of Microbiology, Department of Neurosurgery, Department of Otorhinolaryn-gology, Government Medical College Hospital, Thiruvananthapuram and Department of Oral and Maxillofacial surgery, Government Dental College, Thiruvananthapuram.

Study Period: One year period – September 2016 to August 2017

Study Population: Patients clinically diagnosed with deep space infections of head and neck attending Department of Oral and Maxillofacial surgery, Government Dental College, Thiruvananthapuram, and patients admitted in the Department of Neurosurgery and Otorhinolary-ngology, during the study period.

Sample Size: Calculated using the formula: $N = 4PQ / D^2$ **(N = 130)**

Sampling Technique: Consecutive samples were collected from patients satisfying inclusion criteria.

Data Collection: Using a proforma, data including patient details are collected.

Sample Collection

All aspirated pus samples and tissue specimens collected under aseptic precautions from deep space head and neck infections. Specimens for anaerobic bacteria are collected in air tight bottles preferably filled to the brim or in syringes with the needle sealed with rubber cork.

Processing of Specimens

All samples were processed in the 24 hours Clinical Microbiology laboratory at Government Medical College Hospital, Thiruvananthapuram, immediately after collection. Gram stain, Aerobic culture and anaerobic culture was performed on all specimens. For Aerobic culture the samples were inoculated on to Blood agar, Chocolate agar, MacConkey agar and Brain heart infusion broth and incubated at 37^0C for 48 hours under aerobic conditions. For anaerobic culture, the specimens were initially inoculated into Robertsons Cooked Meat medium and later subcultured on to Brucella blood agar enriched with Haemin and Vitamin K which was incubated at 37^0 C for 48-72 hours under anaerobic conditions. Anaerobiosis was achieved using gaspak and anaerobic jar. Identification of isolates was done by Gram stain, colony morphology and biochemical reactions.

Results

A total of 130 consecutive samples from patients more than 12 years of age, who were clinically diagnosed with deep space infections of the head and neck, attending Department of Oral and Maxillofacial Surgery, Government Dental College Hospital Thiruvananthapuram and Department of Neurosurgery and Otorhinolaryngology, Government Medical College Hospital, Thiruvananthapuram, during a period of one year from September 2016 to August 2017 were included in the study.

Table 1 Gender Distribution

Gender	Number (Percentage)
Male	84 (65%)
Female	46 (35%)
Total	130 (100%)

Table 2 Age Distribution

Age Group	Number (Percentage)
12-20 years	18 (13.8%)
21-30 years	22 (16.9%)
31- 40 years	19 (14.6%)
41- 50 years	**26 (20%)**
51- 60 years	22 (16.9%)
61-70 years	16 (12.3%)
71- 80 years	7 (5.3%)
Total	130 (100%)

Table 3 Culture Positivity

Specimen	Number (Percentage)
Culture positive	83 (64%)
Culture negative	47 (36%)
Total	130 (100%)

Table 4 Sample analysis

Sample	Number (Percentage)
Aspirated pus	126 (97%)
Infected Tissue	4 (3%)
Total	130 (100%)

Table 5 Anatomical site involved in deep space infection

Anatomical Site	Number (Percentage)
Buccal space	**26 (20%)**
Intra cerebral abscess	22 (16.9%)
Anterior Cervical space	17 (13.07%)
Submandibular space	16 (12.3%)
Peritonsillar space	14 (10.7%)
Parotid abscess	13 (10%)
Ludwig's angina	8 (6.1%)
Retropharyngeal space	4 (3.07%)
Vestibular space	3 (2.3%)
Sub masseteric space	2 (1.5%)
Pterygoid space	2 (1.5%)
Sub mental space	2 (1.5%)
Parapharyngeal space	1 (0.7%)
Total	**130 (100%)**

Table 6 Risk factor analysis

Risk Factor	Number (Percentage)
Odontogenic infections	**26 (32.09%)**
Diabetes mellitus	21 (25.9%)
Trauma	18 (22.2%)
Recurrent Pharyngitis	9 (11.1%)
Immunosuppression	4 (4.9%)
Recurrent ear infections	3 (3.7%)
Total	**81 (100%)**

Table 7 Proportion of isolates

Culture	Number (Percentage)
Anaerobic isolates	58 (61.05%)
Aerobic isolates	32 (33.6%)
Microaerophilic isolates	5 (5.2%)
Total	95 (100%)

Table 8 Proportion of monomicrobial and polymicrobial infections

Isolate	Number (Percentage)
Monomicrobial	70 (84.33%)
Polymicrobial	13 (15.66%)
Total	83 (100%)

Table 9 Distribution of Isolates

Isolates	Aerobic Number (Percentage)	Anerobic Number (Percentage)
Monomicrobial	36	54
Polymicrobial	1	4
Total	37 (100%)	58 (100%)

Table 10 Organism wise distribution of aerobic monomicrobial isolates

Aerobic isolate	Number (Percentage)
Streptococcus pyogenes	17 (53.12%)
Staphylococcus aureus	7 (21.8%)
Klebsiella pneumoniae	5 (15.6%)
MRSA	2 (6.25%)
Nocardia farcinica	1 (3.1%)
Total	32 (100%)

Table 11 Organism wise distribution of anaerobic monomicrobial isolates

Isolate	Number (Percentage)
Peptostreptococcus species	37 (63.7%)
Propionibacterium species	12 (20.6%)
Bacteroides species	6 (10.3%)
Lactobacillus species	3 (5.1%)
Total	**58 (100%)**

Table 12 Organism wise distribution of anaerobic polymicrobial isolates

Isolate	Number (Percentage)
Peptostreptococcus + Bacteroides species	2 (50%)
Peptostreptococcus + Propionibacterium species	1 (25%)
Lactobacillus + Bacteroides species	1 (25%)
Total	4 (100%)

Table 13 Sensitivity pattern of anaerobic isolates to Metronidazole

Organism	Sensitive Number (%)	Resistant Number (%)
Bacteroides species (6)	6 (100%)	0
Lactobacillus species (3)	3 (100%)	0
Peptostreptococcus species (37)	35 (94.5%)	2 (5.5%)
Propionibacterium species (12)	10 (83.3%)	2 (16.7%)

Discussion

The present study included 130 patients attending Government Medical College hospital and Dental College Hospital, Trivandrum, with deep space infections of head and neck during the time period September 2016 to August 2017. Patients above 12 years of age were consecutively selected for the study. Patients who presented with superficial lesions and meningitis and those below 12 years of age were excluded from the study. Out of the 130 study population, 84 (65%) were males and 46 (35%) were females. The male : female ratio was 1.8:1. This is similar to the gender distribution reported in the studies conducted by Santhosh et al [5] (males – 63.33%, females – 36.66%) and Rega AJ et al [6] with incidence rate of 54 % in males and 46% in females. A study conducted by Brito TP et al[3] reported 55.5% males and 44.5% females. But female predominance was reported in a retrospective analysis study done by Thimmappa TD et al [7] at Karnataka, India, from January 2009 to January 2015. In the study, out of the total number of 50 patients, 27 (54%) were females and 23 (46%) were males.

Among the 130 samples collected for the study, aspirated pus was collected from 126 cases (97%) from deep space abscesses. In four cases (3%) the infected tissue samples were collected. All the four patients had intra cerebral abscess and two underwent previous neurosurgery procedure. The high incidence of buccal space infection is consistent with other studies in the literature. It is probably due to the predominance of dentoalveolar infections as a major risk factor in deep space infections of head and neck. According to the study conducted by Pourdanesh et al buccal (30.5%) and submandibular (25.04%) spaces were the most affected facial spaces. The study on orofacial infections by Santhosh et al [5] also showed buccal space (66.7%) as the most common space affected. The lower incidence of retropharyngeal and parapharyngeal abscess could be due to prompt antibacterial therapy arresting the spread of infection from other areas. Still, certain studies have shown higher incidence of retropharyngeal and parapharyngeal infections. In the study conducted on neck abscess by Nayak et al out of 44 patients taken up for study, 17 had superficial neck abscess, 13 had retropharyngeal abscess, 11 had Submandibular abscess and four had parapharyngeal abscess. In the study by Thinmappa et al,[7] submandibular space and parapharyngeal space abscess were the most commonly involved with 16 patients (32%) in each. The site most commonly involved according to the study by Brito TP et al[3] was submandibular space in 19 patients (25.6%), followed by the peritonsillar space in 17 patients (22.9%).

The most common risk factor associated with deep space infections of head and neck is odontogenic infections in 26 patients (32.09%) followed by Diabetes mellitus in 21 (25.9%) patients in our study. Trauma due to foreign body or previous surgeries was a risk factor in 18 patients (22.2%). Immunosuppression such as patients on steroids or cancer chemotherapy proved to be a risk factor in 4 (4.9%) patients. Recurrent pharyngitis (9 patients, 11.1%) and ear infections (3 patients 3.7%) also contributed to

deep space infections like peritonsillar abscess and vestibular abscess. 8 patients (6.1%) had multiple risk factors. 49 patients included in the study with deep space infections had no associated risk factors.

Out of the 130 samples collected and processed in the laboratory 64% were culture positive and 36% were culture negative. Majority of the previous studies showed a higher rate of culture positivity. In the study conducted by Kanishka guru et al [8] 91.5% were culture positive and only 8.5% were culture negative. Another study by Brito TP et al [3] reported 84.5% as culture positive and 14.5% as culture negative. Shih-Wei Yang et al reported 89% culture positivity and 11% culture negativity. Among the 83 culture positive cases, 70 (84.33%) yielded monomicrobial growth and 13 (15.66%) yielded polymicrobial growth. Other studies have reported a higher incidence of polymicrobial infections. In the study conducted by Kanishka guru et al [8] polymicrobial infection was found in 55.1% of the specimens. According to the study by Shih-Wei Yang et al, Fifty-one out of 89 positive cultures were polymicrobial (57.3%). The remaining 38 cultures grew monomicrobial pathogen (42.7%).

Among aerobic isolates, the most common pathogen is *Streptococcus pyogenes* in 17 patients (53.12%), followed by *Staphylococcus aureus* in seven patients (21.8%). Methicillin resistant Staphylococcus aureus (MRSA) was isolated from two patients (6.25%), *Klebsiella pneumoniae* from five patients (15.6%). One isolate of *Nocardia farcinica* was isolated from a case of Hodgkins lymphoma on chemotherapy, who presented with brain abscess. Micro aerophilic Streptococcus species were isolated in five cases.These findings were consistent with that of Brito TP et al who reported *S. pyogenes* as the most common microorganism present in 25 patients (23.3%) and *Staphylococcus aureus* (13 patients, 12.1%) [3].

The most common anaerobic pathogen in the study was Peptostreptococcus species in 37 cases (63.7%), followed by Propionibacterium species in 12 patients (20.6%), Bacteroides species in 6 patients (10.3%) and Lactobacillus species in 3 patients (5.1%). Same organisms have been isolated in a similar study conducted by Thimmappa TD et al[7]. Anaerobic Streptococci (41.37 %) was the most common isolate, followed by Bacteroides species (8.63 %) in the study conducted by Fating et al [9].

Among the aerobic isolates, 17 were *Streptococcus pyogenes* (53.12%). All the isolates were sensitive to Penicillin, Erythromycin, Vancomycin and Linezolid. The study conducted by Lee Y Q et al[10] also reported a similar finding. All strains of Streptococcus species in their study were sensitive to Penicillin and Erythromycin. All the *Staphylococcus aureus* isolates, (7) were sensitive to to Gentamicin, Co- trimoxazole, Amikacin, Cefoxitin, Vancomycin, Rifampicin, Clindamycin and Linezolid. 4 isolates (57.1%) were sensitive to Penicillin and 6 (85.7%) were sensitive to Erythromycin. D test was performed and was negative, indicating that there is no inducible resistance to Clindamycin.Two isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA) were obtained from patients admitted with parotid abscess and Ludwig's angina. Both were sensitive to Amikacin, Rifampicin, Clindamycin and Linezolid. Using Vancomycin E-strip method, testing for MIC determination was done and found to be sensitive in both isolates. MIC was less than 1μg/ml. *Nocardia farcinica* was isolated from a Hodgkins lymphoma patient on chemotherapy, who presented with brain abscess. It was found to be sensitive to Cotrimoxazole, Imipenem and Linezolid.

The only gram negative isolate obtained in the study is *Klebsiella pneumoniae* (5). All isolates (100%) were sensitive to Gentamicin, Ceftriaxone, Amikacin, Piperacillin Tazobactam and Cefoperazone Sulbactam. 3 isolates (60%) were sensitive to Cefazolin and Ciprofloxacin. This is in accordance with the study conducted by Lee Y Q et al where all the 26 *Klebsiella pneumoniae* isolates were sensitive to second and third generation cephalosporins and Beta lactam –

beta lactamase inhibitor combinations like Amoxicillin – Clavulanic acid.

Antibiotic sensitivity testing of anaerobes was performed by agar dilution method as per CLSI guidelines. Sensitivity to Metronidazole alone was tested. The isolates which yielded growth in metronidazole concentrations more than 4 µg/ml was reported as resistant. Of the 58 anaerobic isolates obtained in the study, only four (6.8%) were found to be resistant to Metronidazole. Out of the130 cases included in the study, 6 patients died. Among them 3 patients had uncontrolled Diabetes mellitus as an associated risk factor and 2 of them had intracerebral abscess following trauma. The mortality rate in the present study was 5% (6 patients). Mortality rate was high around 54% in the pre antibiotic era, however it is reduced to as low as 4% now due to advanced technology, antibiotic and protocol management.

Conclusion

The study on 'Aerobic and anaerobic bacterial profile of deep space head and neck infections in a tertiary care hospital' was conducted at the Government Medical College, Thiruvananth-apuram during a period of one year from September 2016 to August 2017. Samples were collected from patients attending the Department of Oral and Maxillofacial Surgery, Government Dental College, Thiruvananthapuram, Department of Neurosurgery and Department of Otorhinolaryngology, Government Medical College, Thiruvananthapuram. Patients with age group above 12 years to 80 years were included in the study.A total number of 130 samples were collected under sterile precautions. Majority were aspirated pus samples (97%) and 3% were infected tissue. Processing of samples were done in the 24 hours Clinical Microbiology laboratory immediately after collection. Overall culture positivity was 64%. The culture positivity in aspirated pus sample was 64.2%. Culture positivity among tissue samples was 50%. Out of the 95 isolates obtained in culture, 58 (61.05%) were anaerobic organisms, 32 isolates (33.6%)

were aerobic organisms and five (5.2%) were microaerophilic organisms. Majority of the infections were monomicrobial (84.33%) and polymicrobial infections constituted 15.66%.The most common aerobic organism isolated was *Streptococcus pyogenes* (53.12%) followed by *Staphylococcus aureus* (21.8%), *Klebsiella pneumoniae* (15.6%), Methicillin Resistant *Staphylococcus aureus* (MRSA) (6.25%) and *Nocardia farcinica* (3.1%). The most common anaerobic organism isolated in the study was Peptostreptococcus species (63.7%) followed by Propionibacterium species (20.6%), Bacteroides species (10.3%) and Lactobacillus species (5.1%). Sensitivity testing of anaerobes to Metronidazole was done by agar dilution method according to CLSI guidelines. All the isolates of Bacteroides species and Lactobacillus species were sensitive to Metronidazole. 94.5% of the Peptostreptococcus species were sensitive and 5.5% were resistant to Metronidazole. 83.3% of Propionibacterium species were sensitive and 16.7% were resistant to Metronidazole.Among the polymicrobial infections, 50% of the infections were caused by Peptostreptococcus + Bacteroides combination, 25% by Peptostreptococcus + Propionibacterium species and 25% by Lactobacillus + Bacteroides species. The prevalence of deep space infections (DSI) of head and neck was found to be more in the age group between 41-50 years (20%). Buccal space (20%) was the most common anatomical site involved followed by intra cerebral (16.9%), anterior cervical (13.07%) and Submandibular space (12.3%). The most common risk factor identified was Odontogenic infections (32%) followed by Diabetes mellitus (25.9%). DSI was found to be more among male population (65%) than female population (35%).The mortality rate observed in the study was 5%.

Inspite of the best treatment, the mortality rate of deep space infections of head and neck remains high due to varied reasons. The presence of risk factors such as Diabetes Mellitus can further lead to complications and increased mortality. Aggressive airway maintenance, intravenous

antibiotics and surgical drainage form the cornerstones of management. Although culture-guided antimicrobial therapy is advocated, empirical antibiotics play a critical role in the clinical course of the disease. Being a tertiary care centre, Government Medical college, Thriuvananthapuram, receive more number of cases referred from peripheral centres, in the later stages of the disease. The mortality becomes high due to delay in diagnosis and treatment. Hence, this study was attempted to find out the bacterial profile of deep space infections of head and neck in patients admitted at Government Medical college Hospital, Thiruvananthapuram, and their antibiotic susceptibility pattern, so that empirical antibiotic therapy maybe initiated as early as possible. The mortality rate maybe significantly reduced in future by early diagnosis and intervention.

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