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MELIOIDOSIS-Case series in a tertiary care centre in South Kerala

Microbiology

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ABSTRACT

Melioidosis is a disease caused by Burkholderia pseudomallei restricted to South East Asia and Northern Australia. Occasionally cases are reported from India and China. Recently a good number of cases have been reported from Manipal, Vellore and the Union Territory of Pondicherry particularly within the last 3 years.The important risk factors are uncontrolled Diabetes mellitus, Alcoholism and Chronic Kidney disease. Pneumonia is the commonest clinical presentation spread from the lungs or skin in debilitated individuals gives rise to septicemic forms of melioidosis, that carry a high mortality. Burkholderia pseudomallei is a saprophytic bacteria found deep in the soil and water. Humans and animals are infected by percutaneous inoculation, inhalation and ingestion. Here we report 4 cases of melioidosis confirmed by culture with Dept. of Microbiology at Govt. Medical College, Thiruvananthapuram within a period of 6 months from April 2016 to September 2016. Two cases presented with pneumonia, one case with Breast Abscess and one case with septicemia. Burkholderia pseudomallei was isolated from samples of sputum, pus and blood in these patients. The cases with pneumonia and breast abscess survived. The patient with septicemia was a case of Chronic liver disease admitted with fever. Burkholderia pseudomallei was isolated from 2 blood samples with same antibiotic sensitivity pattern. In spite of treatment with Meropenem the patient expired on the next day. The mortality rate in bacteremia due to this organism i.e. reported to be 100% in various studies.

KEYWORDS:

Burkholderia pseudomallei, pneumonia, septicemia

Introduction

Burkholderia pseudomallei is a facultative intracellular organism. It is a gram negative bacillus first described by Alfred Whitmore & Krishna Swami in 1912. The organism was known as Pseudomonas pseudomallei for many years. In 1992, it was moved to a new genus Burkholderia. Among the pseudomonads, B. Pseudomallei is a perhaps the most virulent organism. Host compromise is not an essential pre-requisite for the disease although many patients have common underlying diseases like Diabetes mellitus or renal failure. It causes a wide spectrum of diseases ranging from asymptomatic infection, abscesses, pneumonia and disseminated disease. It is a significant cause of fatal community acquired pneumonia and septicemia in endemic areas with significant morbidity and mortality.

Case Reports

A total number of 4 culture confirmed cases of Burkholderia pseudomallei from Govt. Medical College hospital, Thiru vanantha puram, South Kerala is reported in this article. Culture and sensitivity of the clinical specimens such as sputum, aspirated pus and blood collected from the patients were performed in the 24 hours Central Microbiology Laboratory attached to the Govt. Medical College Hospital, Thiruvananthapuram.

Melioidosis- Case series

Case No.1: A 59 year old female diabetic patient on Insulin admitted under Respiratory Medicine, at Chest disease hospital at Trivandrum on 23/4/2016 with C/o Cough with purulent expectoration and clinical diagnosis of right upper lobe consolidation. Sputum sample was sent to the central microbiology Laboratory at Govt. Medical College Hospital for culture and sensitivity testing on 23/4/2016. Burkholderia pseudomallei isolated in culture, was identified by morphology and biochemical reactions. The organism was sensitive to ceftazidime and meropenem. The patient was treated with ceftazidime and discharged after 10 days. She was advised to continue co-trimoxazole for 3 months.

Case No. 2: A 20 year old female was admitted with pneumonia on 9/5/2016under Respiratory Medicine at Chest disease hospital at Trivandrum. Sputum sample was sent to the 24 hours Central Microbiology at Govt. Medical College Hospital, Trivandrum. Culture yielded Burkholderia pseudomallei identified by morphology in gram smear, colony morphology and relevant biochemical reactions. The organism was sensitive to ceftazidime. The patient was treated with ceftazidime, recovered and discharged with oral co-trimoxazole after 10 days.

Case No. 3: A 31 year female attended surgery OP with Breast Abscess and was admitted for I& D on 2/7/2016. Pus samples was sent to the 24 hours Central Microbiology Laboratory at the Govt. Medical college Hospital immediately after the I &D procedure. Culture yielded Burkholderia pseudomallei identified by morphology on grams stain, bipolar staining with Methylene Blue colony characterization and relevant biochemical reactions. The organism was sensitive to ceftazidime and other drugs. Patient was treated with Ceftazidime followed by co-trimoxazole and the wound healed well.

Case No. 4: A 62 year old male farmer by occupation alcoholic with chronic liver disease was admitted under surgery unit on 14/9/2016 at Govt. Medical College Hospital at Trivandrum with fever . There was clinical suspicion of septicemia and 2 samples of blood collected and sent to the 24 hours Central Microbiology Laboratory at Govt. Medical College Hospital at Trivandrum. Culture of the both samples yielded Burkholderia pseudomallei with same antibiotic sensitivity pattern. The organism was sensitive to ceftazidime, co-trimoxazole, Imipenem. Meropenem, Ciprofloxacin. The patient was started on Meropenem. But expired on the next day.

Materials and Methods

Collection of Samples

Sputum samples were collected from the patients admitted with pneumonia in a sterile wide mouthed container and sent to the laboratory without any delay.

Pus sample was aspirated with a sterile syringe and needle from the patient admitted with Breast abscess and also pus samples was collected from the same patient using sterile double swabs after I & D procedure, under sterile precautions.

Blood samples (2Nos) were collected by venapuncture under sterile precautions. Each sample of 5 ml of blood collected from 2 different sites at half an hour intervals and directly inoculated into the blood culture bottle containing 50 ml of Brain Heart – Infusion broth and sent to the Microbiology lab immediately after collection.

Processing of Samples

Immediately after receiving the samples in the laboratory using the sputum samples and pus samples, Gram staining was done.

Culture was done on sheep blood agar, chocolate agar and Mac Conkey agar and incubated at 370C.

Blood culture bottles were kept in the BOD incubator after inoculation and incubated at 370C overnight. Next day, subculture was done on sheep blood agar, chocolate agar and Mac Conkey agar.

Identification of the isolates

Blood agar: Heavy growth of minute nonlytic greyish white smooth colonies appear after 24 hours of incubation, Later, the colonies became large, dry, wrinkled with central umbonations after 72 hours of incubation.

Chocolate agar: Similar type of colonies are produced

Mac Conkey agar: Non lactose fermenting colonies appear which on further incubation becomes pale pink, flat, dry and wrinkled with a metallic sheen.

Nutrient agar shows minute greyish white smooth colonies with a slight metallic sheen after 24 hours of incubation which turns to large, flat, dry, wrinkled colonies with central umbonation after 72 hours.

Antibiotic sensitivity testing was performed on Mueller – Hinton agar by Kirby-Bacur method according to CLSI guidelines.

Methodology

Identification of the isolates

Gram staining - showed small, short, gram negative bacilli

Methylene blue staining – showed bipolar staining/(safety pin appearance).

Motility by Hanging drop method: The organism was motile

Biochemical Reactions

- Oxidase Test positive
- Indole production negative
- Triple sugar Iron Agar – Alkaline slant /No change, No Gas/No H2S production.
- Mannitol Motility Agar- Not fermenting Motile
- Citrate utilization test positive
- Urea hydrolysis test Negative
- Nitrate Reduction test positive
- Aesculin Hydrolysis test Positive
- Arginine dihydrolase test Positive

Antibiotic Sensitivity: Testing was done on Mueller Hinton agar according to the CLSI guidelines.

All the 4 isolates have the same antibiotic sensitivity pattern.
Typically resistant to gentamicin (10 µg/disk) and polymyxin B (300µ/disk)

Results

A total No. of 4 culture confirmed cases of Burkholderia pseudomallei reported from Govt. Medical College Hospital, Thiruvananthapuram. Culture of the samples and the antibiotic sensitivity was done in the 24 hours Central Microbiology Laboratory at GMC, Trivandrum.

I. Analysis of clinical samples

Nature of specimen		clinical diagnosis
I case	sputum	Pneumonia
II case	sputum	pneumonia
III case	Aspirated pus	Breast Abscess
IV case	Blood 2 samples	Septicemia

II Age & gender Vs. Clinical diagnosis

Case No.	Age/ Sex	Clinical diagnosis
I	59/F	Rt UL consolidation
II	20/F	Pneumonia
III	31/F	Breast abscess
IV	62/M	Chronic liver disease with septicemia

III. Gender wise analysis

Total No.	Female	Male
4	3 (75%)	1 (25%)

IV. Predisposing factors in meliodosis

Sl.No	Predisposing factor	No. & %
1	Diabetes Mellitus	2 (50%)
2	Chronic alcoholism	1 (25%)
3	Chronic Liver disease	1 (25%)
4	Chronic Kidney disease	1 (25%)
	Total No.	4 (100%)

V. Bacetermic Vs Non bacteremic - Cases of melioidosis

Total No.	Nonbacteremic Cases No & %	Bacteremic No. & %
4	3 (75%)	1 (25%)

VI. Treatment & Outcome

Age/Sex	Clinical diagnosis	Antibiotic given	Outcome
59/F	Pneumonia	Ceftazidime Co-trimozaside	Survived
20/F	Pneumonia	Ceftazidime Co-trimozaside	Survived
31/F	Breast Abscess	Ceftazidime Co-trimozaside	Survived
62/M	Chronic Liver disease with septicemia	Meropenem	Expired

Sl. No	Antibiotics tested	
	Sensitive	Resistant
1	Ceftazidime	Ampicillin
2	Ciprofloxacin	Gentamicin
3	Tetracycline	Amikacin
4	Co-trimoxazole	Polymyxin B
5	Co-amoxiclav	Colistin
6	Piperacillin-tazobactum	Cephalexin
7	Cefoperazone sulbactum	
8	Imipenem	
9	Meropenem	
10	Aztreonam	

ABST pattern of the isolates

All the isolates obtained from sputum, pus and blood samples had the same antibiotic sensitivity pattern

Discussion

Melioidosis in India has been reported since 1991. A series of 28 patients with septicemic meiliodosis over 10 years has been reported from Vellore (Jesudasan 2003). The same tertiary care centre had reported 6 patients with melioidosis (John 1996). 25 cases of melioidosis were reported from Karnataka which included patients from Kerala (Vidyalakshmi 2007). Recently 38 cases of melioidosis have been reported from Union Territory of Pondicherry. With increasing awareness of melioidosis, more cases have been reported from various regions of India. During a period of 6 months from April 2016 to September 2016, 4 cases of melioidosis have been detected and confirmed with culture and sensitivity testing at Govt. Medical College, Hospital, Thiruvananthapuram, Kerala.

Seasonal variation

During the south east monsoon Kerala gets heavy rainfall along with

heavy winds. The association of between rainfall intensity and melioidosis is well documented from epidemic regions with 75% and 85% of prevalence rate during rainy season in Thailand and Northern Australia. The moist and humid conditions during the monsoon may have an exalting effect on pathogenic virulence of the organism. A study by Vidyalakshmi (2010) Manipal reported prevalence rate of 70.4% during the Monsoon Season. In our study, it was 75%. The aerosolization of bacteria from surface water and soil during heavy winds and rain, larger bacteria inoculating dose and infection with more virulent bacteria.

Predisposing factors

Diabetes Mellitus is the most important risk factor for melioidosis and is reported to increase the relative risk of infections by upto a hundred fold in certain age groups in northern Thailand. In our study, the 62 year old man admitted with chronic liver disease and the 59 year old woman admitted with Pneumonia had diabetes mellitus (50%). In Thailand and Australia, the estimated relative risk of melioidosis in diabetic patients has been reported to be 13.1% and 5.9% respectively. Endemic regions have reported varying rates of diabetes: 39% in Darwin study, 56.6% in a study in Malaysia and 60.3% in Thailand. A study by Saravan et al(2010) reported 68% prevalence and Vidyalakshmi et al (2012) 75.8% reported prevalence rate melioidosis in diabetes at Manipal, Karnataka. Chronic alcoholism is the second common predisposing factor in cases of melioidosis. In a study by Saravn et al, the prevalence rate was 28% at Manipal, Karnataka.

Clinical presentation

Pneumonia i.e. the most common clinical presentation in our series (50%). Which correlates with the studies from endemic regions. In the Darwin study from Northern Australia pneumonia accounted for 51% of cases. In Thailand and Malaysia, pneumonia cases accounted for 45% and 42.1% respectively. In India, the prevalence rate was 32.6%. Inhalation is believed to be the mode of acquisition in acute pulmonary meilioidosis, especially during severe weather conditions like tropical cyclones and intense rainfall with winds.

Pulmonary melioidosis often mimic pulmonary tuberculosis both clinically and radiologically and can be differentiated reliably only by isolation of B. pseudomallei in culture. In our case, series, sputum samples collected from pneumonia cases, were subjected to acid –fast staining by Ziehl-Neelsen method. All the smears were negative for acid fast bacilli. In regions where both melioidosis and pulmonary tuberculosis are prevalent, the differential diagnosis should be kept in mind to rule out the presence of acid fast bacilli in sputum samples.

Pneumonia is the most common clinical presentation reported by many studies from endemic regions. In the Darwin study from Northern Australia, pneumonia accounted from 51% of cases. In Thailand and Malaysia, pneumonia accounted for 45% and 42.1% of cases respectively. Inhalation is believed to be the mode of acquisition.

Fever was the most consistent complaint by all the 4 patients admitted with the disease in our institution Melioidosis is known to present as a febrile illness with protean clinical manifestation ranging from localized infection to acute fulminant septicemia.

Abscesses

Melioidosis presenting as abscesses both superficial and deep is not uncommon. There are case reports of splenic abscesses, liver abscesses, prostatic abscesses and psoas abscesses reported from many centres in India. In Thailand, 95% of splenic abscesses are due to B. pseudomallei. In our study, one isolate was obtained from a case of breast abscess. The incidence of prostatic abscess in Australia is reported as 20% B- pseudomallei has been isolated from dental abscesses also.

Bacteremia

In our study, only one patient had bacteremia. Even though, the treatment was started immediately after the isolation of the organism from blood culture, the patient expired the next day.In the Darwin study House Northern Australia, 50% of patients with septicemia due to B. pseudomallei died. In a study by Saravan et al, 28% of cases septizicemic cases died in Karnataka.

Treatment Regimen

The definitive treatment of melioidosis includes an intensive phase of Ceftazidime or Carbapenem for 2 weeks followed by a continuation or maintenance phase of atleast 3 months with co-trimoxazole or doxycycline to eradicate the organism and to prevent relapse. In our case series the 3 nonbacteremic cases presented with pneumonia and breast abscess were treated with Ceftazidime for 14 days followed by Co-trimoxazole for 3 months with regular follow up. The only bacteremic case admitted with chronic liver disease died even though he was started on Meropenem immediately after isolation of B. pseudomallei from blood culture.

Mortality rate

In our case series, out of the 4 cases only one patient died. The mortality rate was 25% studies from Australia and Malaysia reported 14% and 43% mortality rate respectively. In India, studies report the mortality rate of 30% at Pondicherry and 8% in Manipal. In bacteremic patients the mortality rate is quite high.

Summary and Conclusion

Melioidosis is an emerging bacterial infection in India. Within a period of 6 months from April 2016 to September 2016, 4 cases were detected and confirmed with culture at Govt. Medical college Hospital, Thiruvananthapuram. Out of the 4 cases, 3 cases were during monsoon. The prevalence rate was 75% in our study. Two cases presented with pneumonia, one case with breast abscess and one case with bacteremia. In contrast to other studies, here 3 cases are females. The female to male ratio was 3:1. Non bacteremic patients were treated successfully with Ceftazidime and Co-trimoxazole. Bacteremic patient, even though treated with Meropenem, died. Pneumonia was the most common clinical presentation (50%). Diabetes mellitus was the commonest predisposing factor in our series (50%) and chronic alcoholism was the second most common predisposing factor (25%). The mortality rate in our series was 25% in our institution.

The recognition of this bacterium Burkholderia pseudomallei as a potential agent of biological warfare has stimulated interest in the development of a vaccine.

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