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Original Article

Microbiology Section

Incidence Rate and Antibiotic Susceptibility Pattern of *Listeria* Species in High Risk Groups

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ABSTRACT

Introduction: Listeriosis, a bacterial food borne disease caused by *Listeria* spp. leads to mild food poisoning in the healthy individuals and severe systemic disease in immuno-compromised patients, pregnant women and extremes of age. Listeriosis in India largely remains ignored, even though there is an increase in the prevalence worldwide.

Aim: To determine the incidence rate of *Listeria* spp. in clinical samples collected from various immuno-compromised individuals and to study its antibiotic susceptibility pattern.

Materials and Methods: Total 643 clinical samples from high-risk group individuals were tested for *Listeria* spp. using standard culture and identification methods.

Results: The overall incidence rate of Listeriosis in high risk individuals was 4.98%. The incidence rate in pregnant women and women with bad obstetric history was found to be 11.04%. *Listeria* spp. was found in 10.2 % of gastroenteritis cases, which is one of the major concerns in high risk groups. Increased resistance to clindamycin 68.8%, followed by penicillin G 37.5%, erythromycin 31.3% and ampicillin 25 % was found.

Conclusion: In view of the high incidence rate of Listeriosis in our setting, it should be considered as a differential diagnosis in the high risk groups and diagnostic capability for the pathogen needs to be strengthened. Furthermore, increased antibiotic resistance is a cause for concern and the trends need to be monitored.

Keywords: Immuno-compromised patients, *Listeria monocytogenes*, Listeriosis, Pregnancy

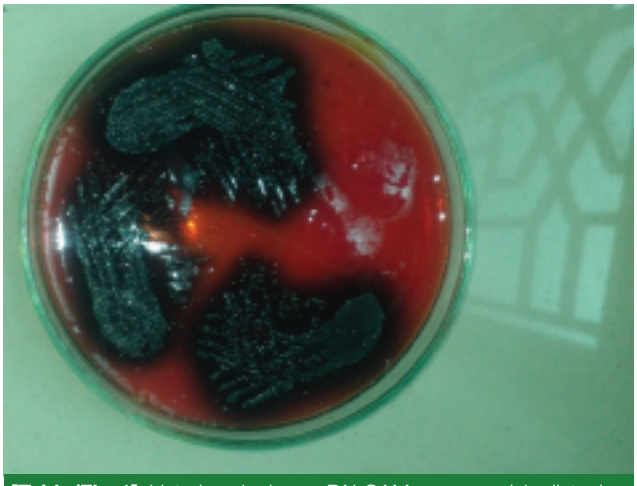
INTRODUCTION

Listeriosis, a potentially serious invasive bacterial food borne disease caused by genus *Listeria* which leads to mild food poisoning in healthy individuals and severe systemic disease in certain well-defined high-risk groups.The genus *Listeria* includes multiple species namely *L.monocytogenes, L.ivanovii, L.innocua, L.fleischmannii, L.welshmeri, L. seeligeri, L.grayi, L.marthii and L.rocourtiae* [1,2]. But among different species only *L.monocytogenes* and *L.ivanovii* are pathogenic in humans [3]. *Listeria monocytogenes* has been found to be the causative agent in several outbreaks of food-borne Listeriosis [4]. *L.ivanovii* infection in humans is although rare, but there are reports on isolation of this organism from cases of AIDS and abortion [5]. Immuno-compromised individuals including transplant patients, dialysis patients, patient on immunosuppressive therapy, HIV patients [6], cancer patients [7], pregnant women [8], infants and neonates [9] are reported to be at high risk of getting Listerial infection. Listeriosis is a serious infection with high case fatality rate of about 20-30%, neonatal death rate 50% and hospitalization rate of about 91% [10]. In view of the high prevalence of *Listeria monocytogenes* in foods, together with the high mortality rate, this pathogen represents an important human health hazard [11]. Reports also suggest that incidence of Listeriosis has been increasing world-wide [12]. Besides, Listeriosis in India largely remains ignored. The literature reviews pertaining to the Listerial infections among immuno-compromised high risk groups in the Indian subcontinent is scarce. *L.monocytogenes* infections are usually treated with a single antimicrobial agent and combined therapies are recommended for the treatment of immuno-compromised patients [13]. Generally penicillin, ampicillin, amoxicillin, cotrimoxazole, tetracycline, chloramphenicol or aminoglycosides are recommended for the treatment of Listerial infection [14]. In 1988, the Multidrug resistant *L.monocytogenes* was first reported in France [15]. Since, then the number of drug resistant strains has been continually increasing [16]. The present study was undertaken to determine the incidence rate of Listeriosis in certain high risk groups. Furthermore, we aimed to determine the drug susceptibility pattern of *Listeria* spp. towards the common antibiotics used in the treatment of Listeriosis.

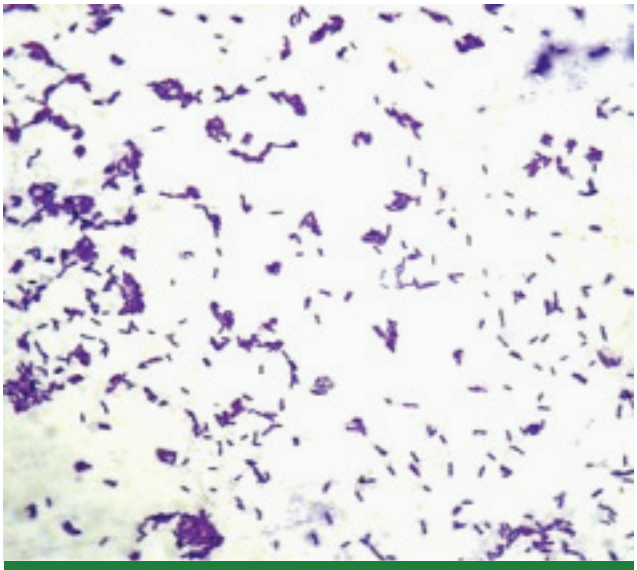
MATERIALS AND METHODS

A cross-sectional study conducted over a period of 1 year from June 2014 to May 2015 in the Department of Microbiology, Sri Lakshmi Narayana Medical College, Hospital and Government General Hospital, Puducherry, India. This study

was approved by the Institutional Human Ethics Committee. Informed consent was obtained from all participants included in the study. Patient population included HIV patients, patients with malignancies, chronic liver disease, chronic renal failure, patients on long term corticosteroid therapy, infants (<12 months of age), pregnant women, women with spontaneous abortions or stillbirths and elderly patients (>65 years) presenting with either fever, flu-like illness, signs and symptoms of meningitis or diarrhea. A total of 643 clinical samples comprising 345 - blood samples, 65 - CSF, 17 - other body fluids, 49- diarrheal stool, 138 - amniotic fluid, 14 - placental bit and 15 - abortus material were aseptically collected. All the collected clinical samples were immediately transported to the laboratory and were processed for the isolation of *Listeria* spp. following the US Department of Agriculture (USDA) method [17]. The samples were enriched by two step enrichment procedure by inoculating in University of Vermont medium (UVM)-1 and incubated at 30°C for 24 hours followed by (UVM)-2 and incubation at 30°C for 24 hours. A loopful of inoculum from enriched (UVM)-2 was cultured on to selective medium PALCAM agar and then incubated at 37°C for 24 hours. Grayish glistening colonies surrounded with a diffuse black zone were identified as *Listeria* colonies [Table/Fig-1] [17,18]. The identified *Listeria* colonies were examined morphologically for Gram positive coccobacilli [Table/Fig-2] and its characteristic tumbling motility at 20-25°C, which was then confirmed for genus *Listeria* on the basis of Latex agglutination test using LK07-Hi *Listeria* Latex Test Kit {Hi-Media, India}. The identified *Listeria* isolates were further subjected to biochemical characterization using KB012A-Hi *Listeria* identification kit which includes catalase test, nitrate reduction test, esculin hydrolysis, Voges-Proskauer test, methyl red test and sugar fermentation tests. The kit contained sugars like xylose, lactose, glucose, alpha-methyl-D mannoside, rhamnose, sucrose and mannitol to differentiate various species of *Listeria*. Isolates exhibiting catalase, methyl red and Voges



[Table/Fig-1]: Listeria colonies on PALCAM agar : grayish glistening with a diffuse black zone showing aesculin hydrolysis.



[Table/Fig-2]: Gram stain showing gram positive coccobacilli (Listeria spp.).

Proskauer test positive and nitrate negative reactions were considered as "presumptive" *Listeria* isolates. These "presumptive" *Listeria* isolates were further differentiated up to the species level into *L. monocytogenes* and other *Listeria* species based on sugar fermentation pattern. Isolates which showed glucose, α-methyl - D mannoside, rhamnose, lactose and sucrose positive, xylose and mannitol negative were considered as *L.monocytogenes*. The recovered isolates were subjected to antimicrobial susceptibility testing by Kirby Bauer disc diffusion assay, as per CLSI guidelines 2012, with the following antibiotics generally recommended for the treatment of the *Listeria* infection such as ampicillin (10 mcg), penicillin G (10 units), tetracycline (30 mcg), chloramphenicol (30 mcg), trimethoprim/sulfamethoxazole (co-trimoxazole) (25 mcg), gentamicin (10 mcg), amikacin (30 mcg), erythromycin (15 mcg) and clindamycin (2 mcg).

RESULTS

Out of 643 samples tested for Listeriosis, 32 samples showed the presence of *Listeria spp*. giving an overall incidence rate of 4.98%. Invasive Listeriosis had an incidence rate of 4.2% compared to Non-Invasive Listeriosis which had an incidence rate of 0.8% [Table/Fig-3]. Abortus material (13.3%) and amniotic fluid (11.6%) yielded the highest rate of isolation followed by diarrhoeal stool (10.2%) [Table/Fig-4]. Of the high risk groups, included in this study the incidence rate was highest among pregnant women and women

Listeriosis type	No. of cases Positive	Positive Percentage (%) n = 643
Invasive Listeriosis	27	4.2
Non-Invasive Listeriosis	5	0.8

[Table/Fig-3]: Percentage of invasive and non- invasive Listeriosis.

Specimen	No. of Samples Processed	No. of samples Positive for Listeria	Positive Percentage (%)
Blood	345	6	1.7%
Amniotic Fluid	138	16	11.6%
Diarrhoeal Stool	49	5	10.2%
CSF	65	2	3.1%
Other Body Fluids (Pleural, Peritoneal, Pericardial, Synovial)	17	0	0%
Placental Bits	14	1	7.1%
Abortus Material	15	2	13.3%
Total no. of Samples	643	32	4.98%

[Table/Fig-4]: No. of specimens positive for *Listeria* spp.

with bad obstetric history (11.04%) followed by the elderly (7.69%) and HIV patients (5.17%) [Table/Fig-5]. Biochemical characterization revealed that (21.9%) of *Listeria* isolates were *L. monocytogenes* [Table/Fig-6]. Highest overall resistance rates among the isolates were to clindamycin (68.8%), followed by penicillin G (37.5%) and erythromycin (31.3%) [Table/Fig-7].

High Risk Individuals	No. of cases	No. of *Listeria* isolates	Percentage (%)
HIV Patients	58	3	5.17%
Pregnant women and women with bad obstetric history	163	18	11.04%
Neonates and Infants	205	3	1.46%
Chronic Renal Failure	56	0	0%
Chronic Liver Disease	52	1	1.92%
Cancer Patients.	31	1	3.22%
Older Adults (>65)	78	6	7.69%

[Table/Fig-5]: *Listeria* spp. isolated from various symptomatic high risk group individuals.

Listeria species	No. of Isolates	Percentage (%) of Strains n=32
L. monocytogenes	7	21.9%
Other *Listeria* spp.	25	78.1%

[Table/Fig-6]: Results of biochemical characterisation of *Listeria*.

Antibiotics	No. of Resistant Strains		Total no. of Resistant Strains n=32(%)
	L. Monocytogenes n=7	Other *Listeria* spp. n=25	
Penicillin G	2	10	12 (37.5)
Ampicillin	1	7	8 (25)
Erythromycin	1	9	10 (31.3)
Clindamycin	2	20	22 (68.8)
Cotrimoxazole	0	4	4 (12.5)
Tetracycline	1	3	4 (12.5)
Gentamicin	0	6	6 (18.8)
Amikacin	0	6	6 (18.8)

[Table/Fig-7]: Antibiotic Resistance Pattern of the isolated *Listeria spp*.

DISCUSSION

In the present study the overall incidence rate in high risk individuals was 4.98%, which is higher compared to previous studies, where incidence rates of only 0.1 % and 11.3/1,000,000 Listerial cases were reported [10,19,20]. This may be because we also included *Listeria* species other than *Listeria monocytogenes* when calculating the incidence rates.The percentage of *L. monocytogenes* in our study was 21.9 %, compared to other *Listeria* spp. which accounted for 78.1%. Further, pathogenicity studies are essential to prove the pathogenic potential of other *Listeria* spp. isolated in this study. Listeriosis was found to be high among pregnant women, older adults and HIV patients. These results are in accordance with the other published reports who reported an increasing incidence of Listeriosis in these groups, especially in elderly population and pregnant women [9,21-27]. In our study, *Listeria* spp. was isolated from 13.3 % of the abortus specimens. This is in accordance with other studies from India which reported a positivity rate of 1.34% - 4% from pregnant women and 14% from women with bad obstetric history [20,28,29]. Maternal Listeriosis is usually mild but it is highly severe and fatal for the neonates [22,30]. Hence, the present study emphasizes the need to screen for Listeriosis in all stages of pregnancy. We found *Listeria* spp. in 10.2% of gastroenteritis cases among high risk groups, whereas another study has reported 20 –25% incidence of *Listeria* spp. in gastroenteritis cases among high risk groups [31]. Listerial gastroenteritis is usually self -limiting in healthy individuals, but it is of major concern in case of immunocompromised individuals.

Incidence of antibiotic resistance was currently low in *L.monocytogenes* compared to other *Listeria* spp. in our study. This study also observed relatively low percentage of tetracycline resistance in *L. monocytogenes* (14.3%), the most widely reported resistance [14,32,33]. Overall increased resistance among *Listeria* genus to clindamycin (68.8%), penicillin G (37.5%), erythromycin (31.3%) and ampicillin (25%) was found. These resistance rates have to be interpreted with caution owing to the less number of isolates. Ampicillin resistance in clinical Listeria has been previously proved due to transfer of plasmids. Moreover increased clindamycin ampicillin and penicillin resistance has also been reported from food and other environmental sources [14,15,32-34], so it is possible that Listeria could have acquired drug resistance genes from multiple sources.

LIMITATIONS

All *Listeria* spp. isolated in the study were taken in to account. Biochemical speciation of non-*Listeria monocytogenes* isolated in this study was not done. Further, pathogenicity studies are required to prove the importance of non - *Listeria monocytogenes* isolated in this study.

CONCLUSION

In view of the increased incidence of Listeriosis, this disease should be considered an important differential diagnosis in clinical practice especially in high risk individuals. Maternal Listeriosis should be considered in all stages of pregnancy and due diligence must be followed in laboratory diagnosis. Being a food borne pathogen, strong efforts have to be made to ensure food safety. The increased drug resistance in this genus to the commonly prescribed antibiotics against Listeriosis is an area of concern and judicious use of antibiotics is to be encouraged to prevent further increase in resistance.

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