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Epidemiology of diabetic foot infections in a reference tertiary hospital in India

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ABSTRACT

Introduction: The present study attempts to examine the microbial profile and antibiotic susceptibility of diabetic foot infections in the intensive care unit of a tertiary referral centre for diabetic foot. As part of the study, we also attempted to find the prevalence of *bla*NDM-like gene among carbapenem-resistant gram negative infections.

Methodology: A prospective study of 261 patients with diabetic foot infections was performed during the period between January 2014 and June 2014.

Results: A total of 289 isolates were obtained from 178 tissue samples from 261 patients, 156 (59.7%) males and 105 (40.2%) females, with a mean age of 58 years (−15 years), having diabetic foot infection (DFI). No growth was seen in thirty eight (17.6%) tissue samples. Out of the total samples, 44.3% were monomicrobial and 55.7% were polymicrobial. Gram negative pathogens were predominant (58.5%). Seven of the total isolates were fungal; 0.7% showed pure fungal growth and 1.7% were mixed, grown along with some bacteria. The most frequently isolated bacteria were *Staphylococcus aureus* (26.9%), followed by *Pseudomonas aeruginosa* (20.9%). Of the 58.5% gram negative pathogens, 16.5% were *Enterobacteriaceae* resistant to carbapenems. Among these isolates, 4 (25%) were positive for blaNDM-like gene. Among the rest, 18.6% were carbapenem-resistant *Pseudomonas*, among which 4 (36.3%) were *bla*NDM. Among the *Staphylococci*, 23.7% were methicillin-resistant *Staphylococcus aureus* (MRSA).

Conclusions: Our results support the recent view that gram negative organisms, depending on the geographical location, may be predominant in DFIs. There is an increase in multidrug-resistant pathogens, especially carbapenem resistance and this is creeping rapidly. We need to be more judicious while using empiric antibiotics.

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Introduction

Foot ulcers and other foot problems are a major cause of morbidity and mortality in people with Diabetes mellitus.[1] Diabetic foot infections (DFIs) are the leading cause of hospitalization for diabetic patients worldwide and in developing countries like India, it accounts for 20% of hospital admissions.[2,3] DFI is a multifactorial process and three factors predispose to tissue damage, namely neuropathy, peripheral vascular disease, and susceptibility to infection whenever there is a direct injury to the foot at risk.[4–6] DFIs are usually polymicrobial, caused by aerobic gram positive cocci like *Staphylococcus aureus*, gram negative bacilli (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*), and anaerobes. Proper management of these infections needs appropriate antibiotic selection.[7] Empirical treatment is based on the pathogens and the susceptibility pattern seen in the community where the hospital is located.

Beta-lactam antibiotics are the most commonly used antibiotics for bacterial infections.[8] However, the accelerated emergence of antibiotic resistance to these groups of drugs among the prevalent pathogens is the most serious threat to the management of such infections, especially carbapenem resistance. These isolates are usually multidrug resistant, which further complicate the scenario.

There is a recent emergence of the NDM metallo-beta-lactamase (MBL) encoding genes among different enterobacterial species and also in non-fermenters like *P. aeruginosa* and *Acinetobacter baumannii* in various parts of world including India.[9–11] In fact, India and Pakistan are the main reservoirs of *bla*NDM-like carrying *Enterobacteriaceae*.[11] There is paucity of data on MBL-producing organisms carrying *bla*NDM-like gene from diabetic foot infections. Taking this into account, we studied the microbial profile and susceptibility pattern of diabetic foot infections in patients with Type 2 Diabetes mellitus to guide empiric therapy for diabetic foot infections in our hospital and the occurrence of *bla*NDM-like carbapenemase gene among carbapenem-resistant gram negative pathogens.

Materials and methods

Study type

A prospective study was performed on 261 diabetic patients with foot ulcers over a period of six months from January 2014 to June 2014. The study was conducted at a tertiary care hospital in Mumbai, India.

Study population

All patients with type 2 diabetes (irrespective of age and sex) who were hospitalized for surgical management of lower-extremity wounds from January 2014 till June 2014 were considered for the study. Their informed consent was obtained and demographic details, duration of lower-limb lesion, duration of diabetes, and type of empiric therapy were documented from their medical records. A deep tissue specimen was obtained from the wounds during surgery and sent for bacterial and fungal cultures.

Specimen collection

After surgical debridement of the slough and necrotic tissue over the wound in the operation theatre, the wound was washed thoroughly with normal saline; a deep tissue specimen of approximately 0.5 × 0.5 cm was taken from the wound bed. The specimen was collected in a sterile container soaked with normal saline and was transported to our microbiology laboratory without delay for further processing.

Specimen processing

Part of the sterile deep tissue specimen was crushed or ground with a sterile mortar and pestle in the biosafety cabinet. The crushed specimen was subjected to gram staining and was streaked on 5% sheep blood agar (SBA), MacConkey agar (MA), and Saboraud's Dextrose agar (SDA) for fungal culture. After inoculation, the SBA was kept in a candle jar and along with MA, it was kept in an incubator at 37 °C. Bacterial isolates and yeast-like fungus identification and susceptibility test was performed using VITEK 2 Compact automated culture system (bioMeriux, France). Commercial I+ IE (imipenem+ imipenem/EDTA) disc from Himedia, Mumbai, India was used for EDTA disc synergy test. All carbapenem resistant *Enterobacteriaceae* were tested for metallobetalactamase production. Modified Hodge test (MHT) was performed for all *Enterobacteriaceae* isolates resistant to carbapenems by Vitek 2 according to CLSI guidelines and EDTA disc synergy test was done for the detection of metallo-β-lactamase production for all gram negative isolates. ESBL production was confirmed by ceftazidime and ceftazidime/clavulunic acid disc synergy test and detection of AmpC β-lactamases was performed as follows.

AmpC detection methods

The isolates were screened for presumptive AmpC production by testing their susceptibility to cefoxitin (30 μg) using Kirby Bauer disc diffusion method and interpreted according to the CLSI guidelines.[12] All the isolates with an inhibition zone diameter of less than 18 mm were labelled as screen positive.

A lawn culture of *E. coli* ATCC 25922 was prepared on MHA plate. A sterile disc of 6 mm moistened with 20 μL of sterile saline was kept and several colonies of test organism were inoculated on this disc. A cefoxitin disc was placed next to this disc (almost touching) on the inoculated plate. The plates were incubated overnight at 37 °C. A flattening or indentation of the cefoxitin inhibition zone in the vicinity of the disc was considered a positive test.[13]

PCR technique for detection of blaNDM

Total DNAs of the different bacterial isolates were extracted by alkaline lysis and PCR to detect blaNDM-like gene was performed as described by Poirel et al.[14]

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