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Prevalence, antibiotic susceptibility and characterization of antibiotic resistant genes among carbapenem-resistant Gram-negative bacilli and yeast in intestinal flora of cancer patients in North Lebanon

Rima Christophy [a], Marwan Osman [a], Hassan Mallat [a], Marcel Achkar [b], Azzam Ziedeh [c], Walid Moukaddem [c], Fouad Dabboussi [a], Monzer Hamze [a,*]

[a] *Laboratoire Microbiologie Santé et Environnement (LMSE), Ecole Doctorale des Sciences et de Technologie, Faculté de Santé Publique, Université Libanaise, Tripoli, Lebanon*
[b] *Clinical Laboratory, Nini Hospital, Tripoli, Lebanon*
[c] *Cancerology Department, Nini Hospital, Tripoli, Lebanon*

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ABSTRACT

The emergence and spread of carbapenem-resistant bacteria are a significant clinical and public health concern. The aim of the study is to determine the prevalence of intestinal carriage of carbapenem-resistant bacteria and yeasts in cancer patients under chemotherapy. 41 stool samples collected from cancer patients in Nini hospital in Tripoli, North Lebanon have been analyzed. After isolating yeasts and carbapenem-resistant bacteria, a biochemical identification and antimicrobial susceptibility profile were determined. The mechanism of enzymatic carbapenem-resistance was detected by searching for carbapenemases by both Hodge test and PCR assays. The association of several mechanisms of resistance was also searched. 46.3% (19/41) of patients were colonized by yeast. *Candida glabrata* (6/19) was the major species. The prevalence of carbapenem-resistant bacteria was 24.4% (10/41) including *Escherichia coli* (5/10), *Enterobacter cloacae* (1/10), *Enterobacter aerogenes* (1/10) *Edwardsiella hoshinae* (1/10) *Pantoea agglomerans* (1/10) and *Pseudomonas stutzeri* (1/10). PCR and sequencing of the amplified fragments revealed that *Pseudomonas stutzeri* (1/1) carried VIM gene and *Enterobacter aerogenes* (1/1) and *E. coli* (1/5) carried OXA-48 gene. The other *Enterobacteriaceae* were resistant to carbapenems by mechanisms other than a carbapenemase including hyperproduction of cephalosporinase (4/10), extended spectrum beta-lactamases (1/10) and both cephalosporinase and extended spectrum beta-lactamases (2/10). High prevalence of intestinal carriage of carbapenem-resistant bacteria and yeasts were detected in cancer patients under chemotherapy. In order to prevent the development of endogenous infection and the dissemination of antimicrobial resistance, an implementation of antibiotic stewardship programs and infection control measures is required in hospitals particularly in the department of chemotherapy.

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Introduction

Cancer patients are at increased risk of infection either because they have a deficiency of the immune function [1] either because of chemotherapy or radiotherapy which induce neutropenia [2]. The prophylactic antibiotic use is a standard practice in patients with neutropenia after chemotherapy [3]. Disruption of the flora of the gastrointestinal tract is then created. In addition, treatment with broad-spectrum antibiotics increases the risk of candidiasis by increasing the concentration of *Candida* spp. in the gastrointestinal tract [4]. The integrity of the mucous membranes that can be damaged by the use of cytotoxic chemotherapy agents also represent a risk factor for the development of fungal infections in these patients [5]. Potentially pathogenic bacteria are able to colonize the intestine which leads to a vicious circle of treatments and the emergence of new bacteria resistant to antibiotics including carbapenem-resistant species [6]. Carbapenems are often used to treat infections caused by enterobacteria producing extended-spectrum β-lactamases (ESBL) or cephalosporinase [7]. They differ from other β-lactams by their post-antibiotic effect against Gram-negative bacteria [8]. Unfortunately, the prevalence of *Enterobacteriaceae* producing carbapenemases increased over the last ten years which seriously leads to a treatment impasse and a challenge in the treatment of nosocomial infections [7]. Two

* Corresponding author.
E-mail address: mhamze@monzerhamze.com (M. Hamze).

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mechanisms of resistance are described, the acquisition of carbapenemases genes that encode enzymes capable of degrading carbapenems or overexpression of ß-lactamases that have a very low affinity for the carbapenems such as cephalosporinase and ESBL trait along with outer membrane porin loss or hyper-expression of efflux pumps [9,10].

On the other hand, the adequate antibiotic therapy is a key issue in multidrug resistant bacteria management. Several studies have shown that cancer patients infected with resistant bacteria more often receive inadequate initial empirical antibiotic therapy, which may impair outcomes, increase mortality and prolong hospitalization [11]. Therefore, the aim of this study was to determine the prevalence of carriage of carbapenem-resistant bacteria and yeasts in the intestinal flora of cancer patients under chemotherapy.

Materials and methods

Samples and data collection

41 cancer patients were included in this study, 28 were suffering from solid tumors and 13 with malignant blood disorders. These patients are treated at Nini hospital in Tripoli, North Lebanon. A standard questionnaire was completed in order to obtain information including the age, gender, type of cancer, date of disease diagnosis, surgical operations in relation to cancer and microbiological history. 41 stool samples were collected between February and July 2014 and transported quickly to the laboratory in a thermostatically controlled container.

Yeast isolation and antifungal susceptibility

Isolation of yeasts was made by culture of stool on Sabouraud agar (*Pronadisa®—Spain*) supplemented with chloramphenicol (0.5 g/l) followed by incubation for 24 h at 37° C. The identification was conducted by the use of Auxacolor™ 2 (*BioRad®—France*). The determination of the sensitivity of different isolates to antifungals was made using Fungitest™ (*BioRad®—France*) according to the manufacturer's recommended procedures.

Isolation of carbapenem-resistant bacteria

Isolation after enrichment method

An amount of stool in the volume of a pea is introduced in a volume of 5 ml of enrichment Tryptic Soy Broth (*Scharlau®—Spain*) supplemented by an antifungal Nystatin (2500 IU/l) (*Medistan®—Lebanon*) and a disc of ertapenem of 10 μg (*BioRad®—France*) and then incubated for 24 h at 37° C. 10 μl of this suspension is then spread on MacConkey agar (*Liofilchem®—Italy*) followed by incubation for 24 h at 37° C [12].

Direct isolation method

An amount of stool in the volume of a pea is introduced in a volume of 5 ml of sterile distilled water. 10 μl of this suspension is inoculated onto MacConkey agar supplemented by ertapenem (*Invanz®—Canada*) with a concentration of 1 mg/l and with Nystatin (2500 IU/L) and then incubated for 24 h at 37° C.

Biochemical identification and resistance pattern

The identification of *Enterobacteriaceae* and oxidase-positive Gram-negative bacilli was carried out through the use of RapID ONE (*Remel®—USA*) and RapID NF (*Remel®—USA*), respectively. The susceptibility to antibiotics of strains resistant to at least one carbapenem was performed by the disk diffusion according to "Comité de l'Antibiogramme de la Société Française de Microbiologie—European Committee on Antimicrobial Susceptibility (CASFM—EUCAST) recommendations.

For the detection of a cephalosporinase, Müller–Hinton agar with cloxacillin (0.25 g/l) was used. Discs of ertapenem, imipenem, meropenem, amoxicillin/clavulanic acid, cefoxitin, ceftazidime, cefotaxime, cefepime and aztreonam were used. This test was repeated on Müller–Hinton agar without cloxacillin. After incubation for 24 h at 37 °C, the diameter of inhibition zones were compared between both Müller–Hinton agar, with and without cloxacillin. For the detection of a carbapenemase, the Hodge test was performed [13]. The same procedure was repeated for *Pseudomonas* strains by using imipenem (10 μg) instead of ertapenem.

Table 1
List of primers used for the detection of ESBL and carbapenemase genes.

Genes	Sequences (5′–3′)	Size (bp)	Refs.
KPC	ATGTCACTGTATCGCCGTCT TTACTGCCCGTTGACGCCC	882	[14]
OXA-48	GGGGACGTTATGCGTGTATT GAGCACTTCTTTTGTGATGGC	900	[15]
NDM-1	GGTGCATGCCCGGTGAAATC ATGCTGGCCTTGGGGAACG	660	[16]
VIM	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	170	[17]
GES	CTATTACTGGCAGGGATCG CCTCTCAATGGTGTGGGT	594	[18]
SME	ACTTTGATGGGAGGATTGGC ACGAATTCGAGCATCACCAG	551	[19]
IMP	GAGTGGCTTAATTCTCRATC AACTAYCCAATAYRTAAC	120	[18]
IMI NMC-A	TGCGGTCGATTGGAGATAAA CGATTCTTGAAGCTTCTGCG	399	[19]
TEM	ATGAGTATTCAACATTTCCG CTGACAGTTACCAATGCTTA	867	[20]
CTX-M	ATGTGCAGYACCAGTAARGT TGGGTRAARTARGTSACCAGA	593	[20]
SHV	GGTTATGCGTTATATTCGCC TTAGCGTTGCCAGTGCTC	867	[20]
OXA	ACACAATACATATCAACTTCGC AGTGTGTTTAGAATGGTGATC	885	[20]
GES	CTTCATTCACGCACTATTAC TAACTTGACCGACAGAGG	827	[20]
PER	GGGACARTCSKATGAATGTCA GGGYSGCTTAGATAGTGCTGAT	827	[20]

Detection of carbapenemases and ESBL genes

Bacterial DNA was extracted by GenElute™ Bacterial Genomic DNA Kit (*Sigma–Aldrich®—USA*). The Protocol followed is the one proposed by the manufacturer. The gene amplification was carried out by the thermal cycler (*Mycycler™ Thermal cycler, Biorad—France*) using the primers described in Table 1.

Results

46.3% (19/41) of patients were infected with yeast. *Candida glabrata* (*C. glabrata*) (6/19) was the major species followed by *Candida albicans* (2/19), *Candida inconspicua* (2/19), *Candida tropicalis* (1/19), *Candida parapsilosis* (1/19) and 7 strains were non identifiable. Table 2 expresses the various antifungal susceptibility profile.

The prevalence of colonization of bacteria resistant to carbapenems in cancer patients under chemotherapy in North Lebanon was

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