Prevalence and resistance to antibiotics of *Enterobacteriaceae* and non-fermentative bacilli isolated at the military hospital specialized in orthopedics at Algiers (2009-2014).

***Aouf Abdelhakim*[1]**, *Rasledjbel Youcef* [2] , *Bakour Rabah*[1] ; [1]*Laboratory of Cellular and Molecular Biology , Faculty of Biological Sciences –University of Sciences and Technologie-Houari Boumediene, Algiers;* [2]*Central Laboratory, military hospital specialized in orthopedics and reeducation- Staouali-Algiers.*

Introduction

Enterobacteriaceae and non-fermentative bacilli are very important microbes in human infectious diseases. From the late 1990s, these groupes of bacteria have aquired resistance to most antibiotics used in therapy, especially to third generation cephalosporins, carbapenems, aminoglycosides, fluoroquinolones and sulphmethoxasole. Multidrug resistant clones have emerged within the community setting as an important cause of a variety of infections. Limitation of drug prescription choice increases therapeutic failure and in consequence the increased mortality rate and an economic burden.

Objective: The aim of this study is to determine the prevalence of *Enterobacteriaceae* and non-fermentative bacilli isolates, to evaluate their level of resistance, and the phenotypic characterization of resistance to carbapenems and third generation cephalosporins and the possibility of horizontal transfer by conjugation. Furthermore a disinfectant (blend of ammonium quaternary compounds) was tested on selected multi-drug resistant isolates. This work was realized at orthopedic, reeducation and reanimation services on hospitalized and non-hospitalized patients.

Methods

The study is a retrospective study jumulated with a prospective study, it was performed on a group of 1482 positive samples ibtained from hospitalized and non-hospitalized patients at military hospital specialized in orthopedics at Algiers from 2009 until 2014. All obtained data are registered on registers and WHONET (laboratory computer system). The growth was quantified and bacteria were identified according to standard procedures. Susceptibility testing was made using disk diffusion method (antibiogramme) and MIC determination according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Mating experiments (Gene transfer by conjugation) were performed as previously described (Bakour et al., 1983) with *E. coli* BM21 (Nalidixic acid resistant) as a recipient. Selective agents were used at the following concentrations: 50 µg/ml for nalidixic acid and 2µg/ml for cefotaxime. Transconjugants were subjected to antibiotics susceptibility. Susceptibility to byotrol was determined by dilution method. *E. coli* ATCC 25922was used as the reference strain for quality control purposes.

Results and discussion

Results obtained are presented in the following tables and figures

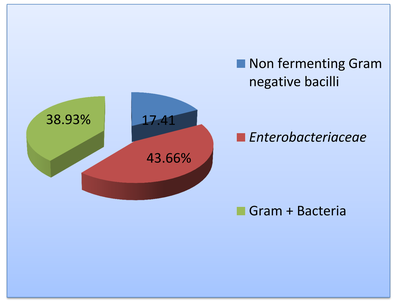

Figure 1: Frequency of isolation according groupes of bacteria.

Table 1: Prevalence of *Enterobacteriaceae* infections by type of samples, services and sexe

Years	Samples			Service			Sexe	
	Urine	Pus	others	Orthopedics	Reanimation	Reeducation	Male	Female
2009	64	93	3	88	0	72	112	48
2010	59	79	3	83	0	58	113	28
2011	41	72	1	75	6	33	91	23
2012	62	39	4	51	9	45	68	37
2013	22	102	3	93	13	21	102	25
Total	248	385	14	390	28	229	479	168
%	12.77	19.83	0.72	20.09	1.44	11.79	24.67	8.65
2014 Jan - May	17	25	5	24	11	12	30	17
%	12.05	17.73	3.54	17.02	7.80	8.51	21.27	12.05

Table 2: Prevalence *P. aeruginosa* infections by type of samples, services and sexe

	Years	Nomber of isolates	Sexe		Type of sample			Service		
			Male	Female	Pus	Urine	others	Reanimation	Reeducation	Orthopedics
Restrospective study	2009	58	51	7	37	9	12	0	7	31
	2010	41	34	7	34	1	6	0	10	31
	2011	28	26	2	22	5	1	1	11	16
	2012	31	19	12	19	6	6	2	11	18
	2013	48	39	9	42	3	3	10	8	30
	Total	206	169	37	154	24	28	13	47	126
	%	100	82	18	75	12	13	7	25	68
Pro Study	2014 Jan - May	36	28	8	27	1	8	10	0	26
	%	100	78	22	75	3	22	28	0	72

Table 3: Prevalence of *A. baumanii* infections by type of samples, services and sexe

Years	Number of isolates	Sexe		Type of samples			Service		
		Homme	Femme	Pus	Urines	Autre	Réanimation	Rééducation	Orthopédie
2011	6	6	0	4	2	0	1	1	4
2012	9	7	2	5	2	2	1	3	5
2013	9	7	2	6	3	0	2	2	5
2014 5 months Jan - May	34	26	8	22	7	5	8	6	20
%	100	76	24	65	20	15	23	18	59

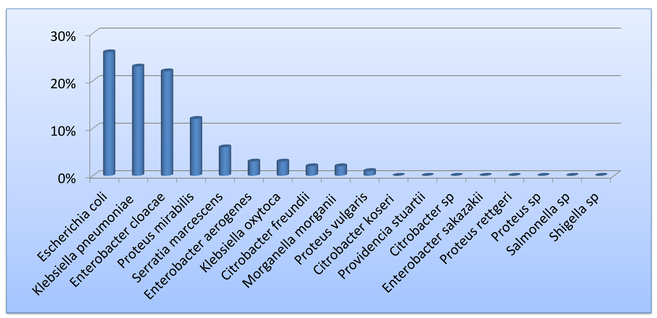

Figure 3 . Resistance profile of *Enterobacteriaceae*

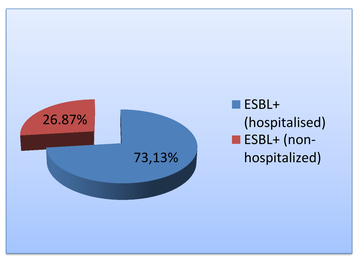

Figure 4 . Frequency of isolation of *Enterobacteriaceae* producing ESBL in hospital and community

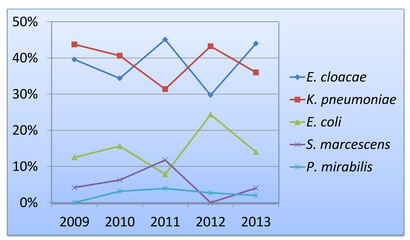

Figure 5: Evolution of resistance of *Enterobacteriaceae* to third generation cephalosporins

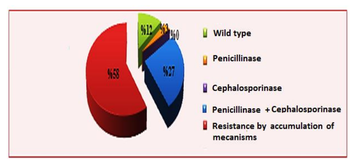

Figure 6: Frequency of resistance mecanismes of *A. Baumanii* to β-lactams

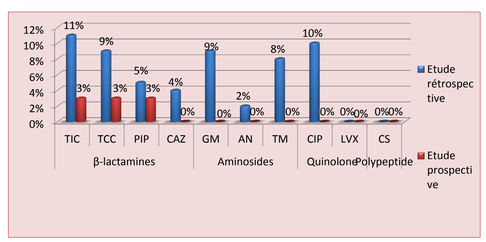

Figure 7: Antibiotic resistance frequency of IMP- Resistant *P. aeruginosa*

On a total of 1482 positive samples, *Enterobacteriaceae* were the predominant (44%), followed by *Staphylococcus* sp. (37%), *Pseudomonas aeruginosa* (13%) and *Acinetobacter baumannii* (4%). Even the low rate of isolation of *A.baumannii* it was responsible of high mortality rate (9%). The frequency of isolation according the sexe show that most isoltes come from males rather than females, this is due mainly to the nature of institution, where most of recruited are males. The most isolated species of *Enterobacteriaceae* were *E. coli, Klebsiella pneumoniae, Enterobacter cloacae, Proteus mirabilis* and *Serratia marsescens*, their predominance depends to the origin of samples. Results of susceptibility to antibiotics showed that isolates have acquired high level of resistance at the exception of *P. aeruginosa*. This resistance concern nearly all antibiotic families used in therapy. Analysis of results showed that the main mechanism of resistance to 3GC in *Enterobacteriaceae* was by ESBL production. *E.cloacae* was the most ESBL producer followed by *K. pneumoniae* and *E. coli*. Resistance of *A. baumannii* to carbapenems was almost due to production of metallo-β-lactamases, and the lost D2 porines. Gene transfer assay showed that ESBL resistance was plasmid mediated in association with other resistance markers especially aminoglycosides, fluoroquinolones, chloramphenicol and sulfamethoxazole. All isolates were sensitive to disinfectant tested.

Conclusion: The increased resistance of *Enterobacteriaceae* and non fermentative bacilli and the dissemination of resistant clones become increasingly great concern in hospitals and in the community in Algeria. Infection caused by ESBL-*Enterobacteriaceae* and IMP-resistant non-fermentative bacilli are respensable for severe diseases for which therapeutic choice is very limited. To reduce the impact of multidrug resistance we must apply strict measures of prevention and control. The judicious choice of antibiotics and disinfectants may reduce consequently the dissemination of multi-drug resistant clones. This critical situation claims an urgent multidisciplinary approach by the cooperation of clinicians, microbiologists and hygienists.