MULTIDRUG RESISTANT BACTERIA IN CHILDREN: OUR WORST NIGHTMARE

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Abstract

Background: Bacterial infections caused by multidrug resistant bacteria (MDR) are a constant challenge for physicians throughout the world. *Aims:* To identify and to phenotype MDR bacteria responsible for infections in children. *Material and methods:* MDR strains isolated from children (0-18 years) admitted at "Louis Turcanu" Children Hospital between April to September 2009 was phenotype at the Microbiology and Virusology Department of University of Medicine and Pharmacy Timisoara. Automated system Vitek ® 2 Compact 30 was used. Methicilino-Resistant Staphylococcus aureus (MRSA) and Methicilino-Resistant Coagulase-Negative (MRCN), extended-spectrum β-lactamase (ESBL)-producing E. coli and Klebsiella pneumoniae, carbapenems-resistance Pseudomonas aeruginosa and Acinetobacter baumanii were studied. *Results:* Out of the 815 bacterial strains isolated during the study period, 23 (2.82%) were MDRS. They were encountered in children admitted in Intensive Care Units (34.78%) and Newborns Departments (34.78%). These bacteria were isolated from bronchial aspirates (30.43%), wound secretions (21.74%), urine cultures (21.74%) and vascular catheters (8.69%) and other specimens. The following phenotypes were identified: PBP mutations in *S. aureus* (13.04%) and *S. coagulase-negative* (21.73%); ESBLs / acquired penicillinase + cephalosporinase, ESBLs or ESBLs (CTX-M) phenotype in *E. coli* (17.39%); ESBLs, ESBLs + Cephamycine impermeability / ESBLs or carbapenems resistance / carbapenemase secretion (metal or KPC) in *Klebsiella pneumoniae* (21.74%) and high-level carbapenems resistance phenotype in *Pseudomonas aeruginosa* (21.74%) and *Acinetobacter baumanii* (4.35%). 34.78% of isolated strains presented extensive drug resistance**.** *Conclusion:* The incidence of MDRS found was not so important. It is necessary to continue the determination of resistance phenotypes, in order to a proper use of antibiotics and to prevent further emergences of MDRS.

Key words: bacteria, drug resistance, infection, antibiotics.

Introduction

Antibiotics have long been considered the "magic bullet" as Paul Ehrlich described them in the 19th century that would end infectious disease. Although they have improved the health of countless numbers of persons, many antibiotics have also been losing their effectiveness since the beginning of the antibiotic era. Bacteria have adapted defenses against antibiotics. They will continue to develop new resistances, even as we develop new antibiotics. In recent years, much attention has been given to the increase of antibiotic resistance. As more bacterial strains become resistant, many infectious diseases have become difficult to treat, a phenomenon frequently ascribed to both excessive and inappropriate use of antibiotics. There is no doubt that the use of antibiotics provides selective pressure responsible for antibiotic resistant bacteria and resistance genes. These strains had a survival advantage, under the selective pressure of antibiotics propagated, and spread throughout the world, contributing to a web of resistance that includes humans, animals and environment.

Nowadays infections caused by multidrug-resistant (MDR) bacteria are daily challenges to physicians throughout the world. During the last decade, the efforts to combat MDR mainly focused on Gram-positive bacteria and drug companies have developed several novel antimicrobial agents to fight these bacteria (e.g. Linezolid or Daptomycin). Unfortunately, the growing problem of MDR in Gram-negative bacteria was not paralleled with the development of novel antibiotics. This explains the growing number of infections caused by Gram-negative bacteria for which no adequate therapeutic options exist. This return to the pre-antibiotic era has become a reality in many parts of the world.

In comparison with Gram-positive bacteria, for which resistance to a single antibiotic indicates the antibiotic resistance phenotype of interest (e.g. Vancomycin-resistant Enterococcus or Methicillin-resistant Staphylococcus aureus), MDR in Gram-negative bacilli are difficult to define. Paterson and Doi[1] introduced the universal definitions for the various degrees of antimicrobial resistance among Gram-negative bacilli. Isolates characterized as MDR are resistant to three or more classes of antibiotics. "Extensive drug resistance" (XDR) defined isolates that are not susceptible to antipseudomonal cephalosporins (Ceftazidime and Cefepime), antipseudomonal carbapenems (Imipenem and Meropenem), Piperacillin/Tazobactam and fluoroquinolones (Ciprofloxacin and Levofloxacin), except Colistin. According to Falagas[2,3] "pan-drug resistant" (PDR) exhibited resistant to all 7 antipseudomonal antimicrobial agents, including Tigecycline and Polypeptide (e.g., Polymyxin B and Colistin).

In order to detect if a bacteria is MDR, its phenotype need tested. A phenotype is defined as the expression of a

specific mechanism of susceptibility or resistance to a given drug class within a particular species[4]. The wild-type phenotype is defined as the phenotype for that species in the "wild," prior to any mutation of chromosomal genes or acquisition of new DNA that alters susceptibility to the drug class.

It is necessary to know the resistance phenotypes existing in your hospital, because they give important information regard to the mechanism of resistance, the emergence and spread of MDR strains. These help to take the correct decision concerning the proper antibiotic treatment.

Objectives

The aim of our study was to identify and to phenotype MDR bacteria responsible for severe infections in children admitted in our hospital.

Material and methods

Our study took place at Emergency Children's Hospital "Louis Turcanu" between Aprils to September 2009. Our lot comprised children (0-18 years) with severe bacterial infections caused by MDR bacteria. We took into consideration the following MDR strains:

- Methicillin-Resistant Staphylococcus aureus (MRSA) and Methicillin-Resistant Coagulase-Negative Staphylococci (MRSCN),
- Extended-spectrum beta-lactamase (ESBL) producing E. coli and Klebsiella pneumoniae,
- Carbapenems-resistance non-fermenting Gram-negative bacilli

After isolation from different specimens received in the Microbiology Laboratory, the strains were tested, in order to identify their antibiotics resistance. The disk-diffusion method was used. MRSA or MRCN defined S. aureus or Coagulase-Negative S. respectively, resistance to Oxacillin and Methicillin. E. coli and Klebsiella pneumoniae resistant to the last generations of Cephalosporins and Aztreonam were ESBL producing strains. Pseudomonas aeruginosa and Acinetobacter baumanii resistant to Carbapenems were also considered as being MDR isolates.

After the identification, all MDR isolates were sent to the Microbiology and Virusology Department of the University of Medicine and Pharmacy, Timisoara in order to phenotype them. Isolates were cultured 24 hours at 37ºC on Columbia agar containing 5% sheep blood and Mac Conkey agar. Suspensions of these cultures were made in 0.45% saline, adjusted to the turbidity of a 0.5 McFarland standard and used to load the cards of system Vitek ® 2 Compact 30. The manufacturer's directions (bio Merieux) were followed. The antibiotic susceptibility test cards were used depending on the type of tested strains. Briefly, for each antibiotic containing test, a turbidity signal was automatically measured at every 15 minutes for up to 18 hours. These data were used to generate growth curves and by comparison with a control, the minimum inhibitor concentration of each antibiotic was estimated. E. Coli ATCC 25922 and 35218, Pseudomonas aeruginosa ATCC 27853 and Staphylococcus aureus ATCC 29213 were used as control strains. These extended antibiograms allow us to fit the tested bacteria into a resistance phenotype.

Results and discussions

During the study period, 815 bacterial strains were isolated and tested in the hospital laboratory. Out of these, 23 isolates (2.82%) fulfilled our inclusion criteria and were considered as MDR bacteria. Gram-negative bacilli (73.81%) were more prevalent than Gram-positive isolates (Table 1). Medical literature showed that Gram-negative organisms have become MDR bacteria over the last two decades and remained to be the major threat all over the world[5].

Table 1: Distribution of isolated bacteria

SPECIES ISOLATED		
	No.	%
Staphylococcus aureus	3	13,04
Staphylococcus hemolyticus	2	8,69
Staphylococcus hominis	3	13,04
E. coli	4	17,39
Klebsiella pneumoniae	5	21,64
Acinetobacter baumannii	1	4,35
Pseudomonas aeruginosa	5	21,74
Total	**23**	**100**

The majority of MDR strains were isolated from bronchial aspirates (30.43%), wound secretions (21.74%), urine cultures (21.74%) and vascular catheters (8.69%) as presented in table 2.

Table 2 Distribution of specimens from which MDR bacteria were isolated.

SPECIMENS	No.	%
Bronchial aspirates	7	30,43
Wounds cultures	5	21,74
Urine cultures	5	21,74
Vascular catheters	2	8,69
Pus cultures	1	4,35
Peritoneal cultures	1	4,35
ETT tubes	1	4,35
Nasal swab	1	4,35
Total	**23**	**100**

As we can see in figure no. 1, the majority of MDR bacteria were encountered in children admitted in intensive care units (ICU) and neonatal departments (ND). Nephrology, Gastroenterology or Pediatric Surgery Department were also other wards in which children were suffering from different infections caused by MDR strains.

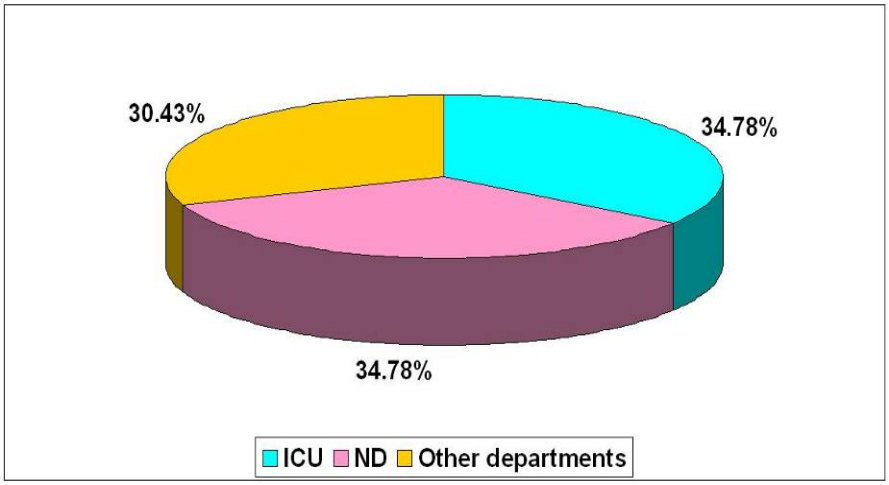

Figure no.1 Distributions of departments with MDR bacteria.

In both ICU and ND, children are exposed to several invasive procedures such as: tracheal intubations, mechanical ventilation, airway aspiration, indwelling vascular catheters in order to save their life. These invasive maneuvers alter their skin or mucous membrane barriers and represent an important pathway for bacteria to enter in the organism and to cause infections. Especially neonates are more susceptible to infections because of their weak immune system and inadequate development of mechanical barriers such as skin and gastrointestinal tract mucosa[6] . Compared with older children and adults, infants, particularly premature infants, are relatively immunocompromised. Small gestational age, low birth weight, mechanical ventilation therapy and prolonged hospitalization were found to be also important risk factors for MDR bacteria infections[7]. Widespread use of broad-spectrum antibiotic in IC and ND is a serious risk factor for the emergence and spread of MDR pathogens[8]. Antibiotic used interferes with colonization by normal flora, thereby facilitating colonization with more virulent pathogens. Studied demonstrated that the rise of E. coli and Klebsiella pneumoniae ESBL strains and Pseudomonas aeruginosa carbapenems-resistant was correlated with increased consumption of extended-spectrum cephalosporins, β-lactam/β-lactamase inhibitor combinations, carbapenems, quinolones and aminoglycosides. Carbapenems-resistant Acinetobacter spp. was significantly associated with the increased usage of extended-spectrum cephalosporins[9]. This flora is frequently MDR as it has developed under the selective pressure of antibiotics and can cause invasive disease. Transmission of pathogenic agents is by direct contact or indirectly either via contaminated equipments, intravenous fluids, medications, blood products or enteral feedings. Various studies have also reported length of stay in ICU or ND as a conditioning factor for the occurrence of infections with severe MDR bacteria. Mean time between ICU admission and acquired of infection with MDR bacteria was 12 +/- 9 days[10, 11].

Table 3 The Gram positive MDR bacteria phenotypes identified.

Species	MDR Gram positive bacteria		
	No. %	Identified phenotypes	No.%
Staphylococcus aureus	3 (13.04%)	**MRSA:**PBP mutations (penicillin-binding protein); Amg-resistant; Fq, glycopeptide, oxazolidinone, fosfomycin, fusidic acid, rifampicin, SXT, tetracycline- wild;	2 (66.66%)
		MRSA: PBP mutations (penicillin-binding protein); Amg-resistant; Fq -partial resistant/ wild; SXT-resistant; MLSB, glycopeptide, oxazolidinone, fosfomycin, fusidic acid, SXT, rifampicin, tetracycline-wild;	1 (33.33%)
Staphylococcus hominis	3 (13.04%)	**MRSCN:** PBP mutations (penicillin-binding protein); Amg-resistant; Fq-resistant /partial resistant ; Fosfomycin, SXT, tetracycline-resistant; MLSB – constitutive; Glycopeptide, fusidic acid, oxazolidinone, rifampicin-wild;	1 (33.33%)
		MRSCN: PBP mutations (penicillin-binding protein); Amg-heterogenous; Fq-partial resistant; Tetracycline- altered targed /partial resistant; Teicoplanin, fusidic acid, rifampicin-resistant; SXT - resistant/wild; Fosfomycin, oxazolidinone-wild;	1 (33.33%)
		MRSCN: PBP mutations; Amg- resistant; Fq- partial resistant; Tetracycline - altered targed/ partial resistant; Rifampicin, SXT-resistant; Fusidic acid, glycopeptide, oxazolidinone, fosfomycin-wild	1 (33.33%)
Staphylococcus haemolyticus	2 (8.69%)	**MRSCN:** PBP mutations (penicillin-binding protein) ; Amg-heterogenous; Fq resistant/partial resistant; MLSB-inductible/resistant; Tetracycline - altered targed/partial resistant; Fosfomicyn, fusidic acid, SXT -resistant; glycopeptide, oxazolidinone, rifampicin-wild;	1 (50%)
		MRSCN: PBP mutations (penicillin-binding protein) ; Amg-heterogenous; Fq resistant/partial resistant; MLSB-inductible/resistant; Tetracycline - altered targed /partial resistant; Teicoplanin, fosfomicyn, SXT- resistant; Oxazolidinone, fusidic acid, rifampicin-wild;	1 (50%)

Note: MRSA- Methicillin-Resistant Staphylococcus aureus; MRSCN- Methicillin-Resistant Coagulase-Negative Staphylococcus; MLS- Macrolide, Lincosamine, Streptomycine; Amg-Aminoglycosides; Fq-Fluoroquinolones; SXT-Trimethoprim/Sulfamethoxazole

Further, it is necessary to know the mechanisms involve in the emergence and spread of MDR strains for a better understanding of resistance phenotypes encountered in our hospital

S. aureus is a Gram-positive bacteria that colonizes the skin of about 30% of healthy humans. Although mainly a harmless colonizer, it can cause severe infection, due in part to its ability to acquire and express an extensive array of virulence factors and antimicrobial resistance determinants. Mobile genetic elements are involved in the dissemination of virulence and resistance genes in S. aureus and include plasmids, bacteriophages, pathogenicity islands, transposons and chromosomal cassettes [12,13]. Its oxacillin resistant form (MRSA) is one of the most important causes of antibiotic resistant worldwide. Moreover, infections with MRSA may result in prolonged hospital stay and higher mortality[14]. According to the European Antimicrobial Surveillance System[15], MRSA proportions varied from less than 1% in the north to over 50% in southern European countries. Romania showed a significant decrease in MRSA proportions in the last four years with an identified proportion of 33%. Although Coagulase-negative Staphylococci (CNS) are commensally bacteria of human skin and nasal mucosa, in the advent of increased invasive interventions and treatments, they have been frequently detected as a cause of opportunistic infections. Predominant CNS species associated with clinically relevant infections are Staphylococcus epidermidis and Staphylococcus haemolyticus. They are difficult to eradicate, as they possess the capacity to form biofilms on indwelling devices. Worldwide surveys revealed that 60 to 85% of clinical strains are resistant to Methicillin [16, 17]. Although there are three known mechanisms for which S. aureus and CNS becomes resistant to Methicillin- hyperproduction of β-lactamases[18], the presence of an acquired penicillin-binding protein and PBP2a[19], and modification of normal penicillin-binding proteins (PBPs)[20] - the last one was responsible for the emergence of resistant strains isolated in our study. The numbers of MRCNS isolates found were higher than MRSA, which was in agreement with other previous works[21]. All MRSA strains were also aminoglycoside-resistant Staphylococci contained at least one aminoglycoside modifying enzyme gene. Other antibiotic resistance phenotypes associated to MRSA and MRSCN are presented in the table 3. Fortunately, no Vancomycin Resistant Staphylococcus was encountered.

Table 4 The Gram negative MDR bacteria phenotypes identified.

Species	Gram-negative MDR bacteria		
	No. %	Identified phenotypes	No.%
E. coli	4 (17.39%)	**ESBL (acquired penicillinase + cephalosporinase);** Amg -heterogenousous; Fq-resistent-1/wild; SXT, Polypeptide- wild;	1 (25%)
		ESBL (acquired penicillinase + cephalosporinase); Amg-heterogenousous; Fq, SXT- resistant; Polypeptide- wild; *	1 (25%)
		ESBL (CTX-M); Amg, Fq, Tetracycline, SXT-resistant; Polypeptide-wild;	1 (25%)
		ESBL; Amg-heterogenous; Fq-resistant-1/wild; Tetracycline, Furan, SXT-resistant;	1 (25%)
Klebsiella pneumoniae	5 (21.64%)	**Carbapenems-Resistant** *(*KPC or metallo-β-lactamases); Amg-heterogenous; Fq, SXT-resistant; Polypeptide-wild; *	1 (25%)
		ESBL+ Cephamycine impermeability / ESBL; Amg-heterogenousous; Fq-wild/resistant-1; SXT-resistant; Polypeptide -wild;	1 (25%)
		ESBL; Amg-heterogenous /wild; Fq-resistant; Furan, Tetracycline-wild/resistant; SXT-wild;	1 (25%)
		ESBL; Amg-heterogenous/resistant; Fq, Tetracycline-resistant; Polypeptide -wild;*	1 (25%)
		ESBL; Amg-heterogenous; Fq-wild/resistant-1; Furan-wild/resistant; Tetracycline-resistant; SXT-wild;	1 (4.35%)
Acinetobacter baumannii	1 (4.35%)	**Carbapenems-Resistant *(*Carbapenemases)**; Fq, Amg, SXT-resistant; Polypeptide-wild;*	1 (100%)
Pseudomonas aeruginosa	5 (21.74%)	**Highly resistant to carbapenems;** Amg, Fq-resistant; SXT-Polypeptide-wild; *	4 (80%)
		Highly resistant to carbapenems/ESBL; Amg, Fq, Polypeptide- resistant; SXT-wild;	1 (20%)

Note: ESBL- extended-spectrum beta-lactamases; Amg-Aminoglycosides; Fq-Fluoroquinolones; SXT-Trimethoprim/Sulfamethoxazole;
*Strains considered as being XDR (N=8)

Nowadays MDR Gram-negative bacilli have become the most important cause of severe hospital and community acquired infections, as well as the consequences with respect to mortality, hospital length of stay and increased hospital costs[22].

ESBL strains have been increasingly reported in Europe since their first description in 1983. These strains confer bacterial resistance to all β-lactams except carbapenems and cephamycins, which are inhibited by other β-lactamase inhibitors such as clavulanic acid. A shift in the distribution of different ESBLs has recently occurred in Europe, with a dramatic increase of CTX-M enzymes over the classical TEM and SHV variants. Although ESBLs still constitute the first cause of resistance to β-lactams among Enterobacteriaceae, other "new beta-lactamases" conferring resistance to carbapenems, such as metallo- β -lactamases and KPC carbapenemases or to cephamycins, such as CMY enzymes[23].

Among the MDR Enterobacteriaceae isolated, 77.77% of isolated strains were ESBL strains. As we can observe in table 4, some strains possess also a penicillinase phenotype after they had acquired a plasmid-mediated penicillinase. Therefore, they are resistant to penicillins and perhaps to Cephalothin. If the strain acquires a plasmid-mediated AmpC β -lactamase from an organism such as Enterobacter spp. or Citrobacter freundii, the strain will have a cephalosporinase phenotype and will display resistance to virtually every β–lactam drug except the carbapenems. Resistance to fluoroquinolones, Tetracycline and Trimethoprim/Sulfamethoxazole were found among our isolates. Thus, the presence of an ESBL is a good marker of the MDR phenotype[24].

Reports of carbapenemases have been increasing over the last few years. This phenotypic grouping of enzymes is heterogeneous mixtures of β-lactamases belonging to molecular Ambler class A (penicillinases), class B (metalloenzymes) and class D (oxacillinases). These enzymes have the common property of hydrolyzing, at least partially, carbapenems together with other penicillin or cephalosporin antibiotics. Out of these, IMP and VIM series in Pseudomonas aeruginosa and Enterobacteriaceae and of the oxacillinase type in Acinetobacter baumannii are clinically worrying [25].

More than half of phenotypes of Gram-negative bacilli tested using Vitek® 2 Compact 30 were resistant to the antipseudomonal cephalosporins, antipseudomonal carbapenems, β-lactam/ β-lactamase inhibitor combinations, fluoroquinolones and aminoglycosides. According to

Falagas[3,4] these phenotypes are characteristic to XDR bacteria. These strains were isolated from children diagnosed with ventilator-associated pneumonia (VAP). Prior antibiotic exposure (3rd generation Cephalosporins), excess antibiotic use and durations of antibiotic administration beyond 7 or 8 days in mechanically ventilated children have been linked with severe infections caused by MDR bacteria[26]. Medical studies recommended the use of Colistin and Tigecycline as the optimal antibiotics in the treatment of XDR infections[27,28]. Tigecycline, the first representative of the glycylcycline class, was approved its use for complicated intraabdominal and skin infections. Regarding its mechanism of action, this antibiotic enters bacterial cells through energy dependent pathways or with passive diffusion[29], but unfortunately, it is not recommended for children use[30] . Colistin was used for about two decades after its discovery in 1950, but the reported nephrotoxicity and neurotoxicity led to gradual decrease of its use. However, this antibiotic recently regained some popularity in several countries as a salvage antimicrobial agent against MDR bacteria. Clinical studies showed the efficiently of inhaled Colistin in treating VAP caused by MDR bacteria[31,32], but unfortunately, a relationship between the increasing clinical use of Colistin and resistance in Gram negative bacilli has been reported. Colistin-resistant Pseudomonas aeruginosa, Acinetobacter baumannii have been described [33, 34, 35]. Hence, this antibiotic should be spread for serious cases in order to avoid the emergence of MDR or even PDR bacteria.

Conclusions

The purpose of this article was to present new information concerning the MDR bacteria and to underline the importance of knowing the resistance phenotype in a hospital. The resistance phenotype helps in choosing the best antibiotic treatment in order to cure the infection and to prevent the emergence and spread of new MDR strains. Although old pathogens continue to be a threat around, new and more powerful, MDR bacteria emerge under the selective antibiotics pressure.

The incidence of MDR isolates encountered in our hospital was not so important (2.82%). While approaching the "end of antibiotics era" new strategy need imposed. It is easy to prevent than to fight with MDR bacteria. Older preventive healthcare measures such as hand hygiene and vaccinations against the most common bacteria during childhood limit the spreading of MDR strains. Hand hygiene" is considered worldwide to be the cornerstone of MDR bacteria infection prevention. In addition, decrease antibiotic consumption is associated with decreased resistant rates of MDR bacteria. De-escalation of the administered antibiotics is required as soon as culture results are obtained.

At the beginning of the 21-century a new question appeared. Do antibiotics continue to be the "magic bullet" or become boomerangs?

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