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Looking Back To Old, Forgotten Antibiotics to Treat Gram Negative Superbugs

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Abstract
Antibiotic resistance has emerged as one of the greatest global health challenges to be addressed in the 21st century.The reintroduction of previously used antibiotics active against extensively drug-resistant (XDR) bacteria represents a new alternative for the control of antimicrobial resistance. A total of 100 extensively drug resistant Gram negative bacilli isolated from Clinical Microbiology laboratory.Acinetobacter was found to be the most common and highly resistant organism in this study (54%), which was isolated mostly from respiratory and pus samples.Other extensively drug resistant Gram negative bacilli isolated from Clinical samples includes Klebsiella (33%) and Pseudomonas(13%).From the 100 Extensively drug resistant gram negative bacteria, the most common respiratory pathogen Acinetobacter shows 78% sensitivity towards Minocycline followed by Klebsiella (21%). Pseudomonas shows complete resistance towards this drug.The old antibiotic Fosfomycin was active against all three species (Acinetobacter 94%, Klebsiella 100% and Pseudomonas 77%).Chloramphenicol shows less sensitivity towards Klebsiella (33%) and Acinetobacter(22%) and pseudomonas(16%).97% isolates were sensitive to polymyxins\colistin.
Keywords: Antibiotic resistance, Gram negative bacilli,Acinetobacter , Klebsiella, Minocycline.

Introduction

Infections and infectious diseases are a great burden on the lives and well being of people around the world. Antibiotics and similar drugs, together called antimicrobial agents, have been used to treat patients who have infectious diseases. Since the 1940s, these drugs have greatly reduced illness and death and their introduction represents a remarkable success story. However, the extensive use and misuse of antibiotics have resulted in selection and world wide spread of antibiotic resistant bacteria. Antibiotic resistance has emerged as one of the greatest global health challenges to be addressed in the 21st century. It dramatically reduces the probability of effectively treating infections and increases the morbidity and mortality associated with common bacterial diseases.

During the last few decades, the incidence of multidrug resistance (MDR) microbial infections

has increased dramatically. Continuous deployment of antimicrobial drugs in treating infections has led to the emergence of resistance among the various strains of microorganisms.[1-3]According to WHO, these resistant microorganisms (like bacteria, fungi, viruses, and parasites) are able to combat attack by antimicrobial drugs, which leads to ineffective treatment resulting in persistence and spreading of infections. Almost all the capable infecting agents have employed high levels of multidrug resistance (MDR) with enhanced morbidity and mortality; thus, they are referred to as "super bugs."

The increasing prevalence of hospital and community-acquired infections caused by multidrug-resistant (MDR) bacterial pathogens is limiting the options for effective antibiotic therapy. Moreover, this alarming spread of antimicrobial resistance has not been paralleled by the development of novel antimicrobials. The problem of increasing antimicrobial resistance is even more threatening when considering the very limited number of new antimicrobial agents that are in development[2]. Of the MDROs, highly-resistant Gram-negative bacteria (e.g. multidrug-resistant carbapenemase-producing Klebsiella pneumonia and Acinetobacter spp.) require special mention; these organisms can be resistant to all currently available antimicrobial agents or remain susceptible only to older, potentially more toxic agents such as the polymyxins, leaving limited and suboptimal options for treatment[14-15].In this context, the rational use of older antibiotics could represent an alternative to the treatment of MDR bacterial pathogens.

Old and new antibiotics vary in their impact on the emergence and spread of resistant bacteria. It is alarming that although bacterial resistance continues to emerge, the rate at which antibiotics are being developed is decreasing. Bacterial strains resistant to newly developed antibiotics also have emerged recurrently. In this context, the reintroduction of previously used antibiotics active against extensively drug-resistant (XDR) bacteria represents a new alternative for the control of antimicrobial resistance. As old antibiotics have rarely been subjected to contemporary drug-development procedures or compared to commonly used antibiotics, they are less considered in practice guidelines. Therefore, their efficacy and safety must be revaluated to optimize therapy. [4]

Large amounts of antibiotics used for human therapy, resulted in the selection of pathogenic bacteria resistant to multiple drugs. Multidrug resistance in bacteria may be generated by one or two mechanisms. First, these bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation occurs typically on resistance (R) plasmids. Second, multidrug resistance may also occur by the increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs. Many different definitions for multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) bacteria are being used in the medical literature to characterize the different patterns of resistance found in healthcare-associated, antimicrobial resistant bacteria. MDR was defined as acquired

non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR was defined as non-susceptibility to all agents in all antimicrobial categories.[5-7]

Many of the bacterial pathogens associated with epidemics of human disease have evolved into multidrug-resistant (MDR) forms subsequent to antibiotic use.The term "superbugs" refers to microbes with enhanced morbidity and mortality due to multiple mutations endowing high levels of resistance to the antibiotic classes specifically recommended for their treatment; the therapeutic options for these microbes are reduced, and periods of hospital care are extended and more costly. In some cases, super resistant strains have also acquired increased virulence and enhanced transmissibility. Realistically, antibiotic resistance can be considered a virulence factor. Bacterial strains resistant to newly developed antibiotics have emerged recurrently. Therefore, antimicrobial resistance presents an ongoing challenge that requires a multifaceted approach including (i) biomedical innovation, (ii) improved surveillance of antibiotic consumption and antimicrobial-resistance rates, (iii) prevention of health-care- associated infections and transmission of multidrug-resistant (MDR) bacteria and environmental dissemination, (iv) rapid microbiological diagnosis, and (v) curtailed clinical and veteri- nary misuse. It is alarming that although bacterial resistance continues to emerge, the rate at which antibiotics are being developed is decreasing.

In this context, the reintroduction of previously used antibiotics active against XDR bacteria represents a new alternative for the control of antimicrobial resistance . Treatment of infections caused by pan resistant Acinetobacter, Enterobacteriaceae and Pseudomonas was proving a challenge to clinicians, particularly in the intensive care scenario.[3] Older drugs such as colistin, chloramphenicol, minocycline and fosfomycin were reconsidered, either alone or in combination with newer agents.

Minocycline is a highly lipophilic semisynthetic second-generation tetracycline antibiotic.The tetracyclines are primarily bacteriostatic and are thought to exert their antimicrobial effect by the inhibition of protein synthesis. Minocycline is active against many tetracycline resistant strains of organisms such as staphylococci, streptococci and E. Coli. Fosfomycin is a broad-spectrum antibiotic, with moderate activity against numerous bacterial species.It inhibits bacterial cell wall biosynthesis by acting on the initial stages in the synthesis of peptidoglycan procedures. The target of antimicrobial activity of colistin is the bacterial cell membrane. In addition to the direct antibacterial activity, colistin has also potent anti-endotoxin activity. Colistin has excellent bactericidal activity against most gramnegative aerobicbacilli, including Acinetobacter species, P. aeruginosa, Klebsiella species, Enterobacter species, Escherichia coli, Salmonella species, Shigella species, Citrobacter species,Yersinia

pseudotuberculosis, Morganella morganii, and Haemophilus influenzae. Chloramphenicol inhibits protein synthesis and is active against a wide variety of gram positive and gram negative bacteria.

In view of the alarming spread of antimicrobial resistance in the absence of new antibiotics, this study aimed at assessing the availability and the effectiveness of potentially useful older antibiotics such as Minocycline, Fosfomycin, Colistin and Chloramphenicol as an alternative treatment option for XDR gram negative bacterial pathogens . This study also undertaken to identify the most predominant and extensively drug resistant gram negative organisms isolated from different clinical samples. A total of 100 Extensively drug resistant (XDR) Gram negative bacilli are isolated from different samples (Respiratory sample, Pus, Other body fluids,Urine blood) received from the hospital.Samples are processed and identified by standard laboratory technique .[13]The XDR gram negative bacilli are subjected to study Antimicrobial sensitivity testing for Minocycline, Fosfomycin, Colistin, and Chloramphenicol on Mueller Hinton Agar plates with commercially available discs (Hi-Media&Cipla, Mumbai) by Kirby Bauer Disc diffusion method. The results are recorded and interpreted as per CLSI guidelines.

A total of 100 extensively drug resistant Gram negative bacilli were isolated from Clinical Microbiology laboratory. Age and sex distribution of patients from whom XDR GNB were isolated is shown in table 1. The highest number of XDR GNB were isolated in the above 60 age group.Acinetobacter was found to be the most common and highly resistant organism in this study (54%), which was isolated mostly from respiratory and pus samples. Acinetobacter spp. was most frequently isolated from sputum (46%, i.e. 25 isolates) followed by pus (31%, i.e. 17 isolates), Blood (13%, i.e. 7 isolates) and urine samples (10%, i.e. 11 isolates).Other extensively drug resistant Gram negative bacilli isolated from Clinical samples includes Klebsiella (33%) and Pseudomonas(13%).Table 2 shows the isolation of XDR Gram negative bacilli isolated in various clinical samples.

In the present study, Acinetobacter shows 78% sensitivity to Minocycline whereas Klebsiella species shows only 21% sensitivity. Pseudomonas is totally resistant to Minocycline. A study by Rebolledo et al, [8] Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, Georgia, USA reported that none of the Tetracyclines tested were active against Pseudomonas species.

In the current study, Fosfomycin shows high sensitivity towards all XDROs (Acinetobacter 94%, Klebsiella 100% and Pseudomonas 77%). L. V. Perdigao-Neto et al,[9] Department of Infection Control of Hospital das Clínicas, University of Sao Paulo, Brazil also said that Fosfomycin presented activity against multiresistant microorganisms in their study.

The treatment of emerging XDR gram negative organisms is a challenge. The development of newer antibiotics has recently slowed down. This has led to the re emergence of the ‘old, forgotten’

antibiotic Colistin. In this study, it shows that 97% XDR isolates were sensitive to Colistin which is similar to the study conducted by Sadia Zafar et al [10] where Polymyxin inhibited 98% of isolates.

"Bad Bugs and No Drugs" situations have forced physicians to reintroduce forgotten antibiotics in clinical practice. Chloramphenicol is one such old broad- spectrum antibiotic. This study shows that 33% of the Klebseilla and 16% of the XDR Pseudomonas isolates to be sensitive to Chloramphenicol which is similar to a study done by SmithaSood et al. [11] Acinetobacter species shows 22% sensitivity in the current study. In another study, PurvaMathuret al [12] AIIMS, Delhi found that 20 (7%) isolates of A.baumannii, 24 (8%) P.aeruginosa, 54 (27%) K. pneumoniae, were Chloramphenicol susceptible.Table 3 shows the sensitivity pattern of XDR Gram negative bacilli to old antibiotics such as Minocycline,Fosfomycine,Chloramphenicol and Colistin.

Acinetobacter was found to be the predominant and highly resistant organism in this study (54%).The reintroduction of previously used antibiotics to treat XDR bacteria represents a new alternative for the control of antimicrobial resistance. 97% isolates were sensitive to polymyxins\colistin.The old antibiotic Fosfomycin was active against all three species (Acinetobacter 94%, Klebsiella 100% and Pseudomonas 77%). From the 100 Extensively drug resistant gram negative bacteria, the most common respiratory pathogen Acinetobacter shows 78% sensitivity towards Minocycline followed by Klebsiella (21%). Pseudomonas shows complete resistance towards this drug. Chloramphenicol shows less sensitivity towards Klebsiella (33%) and Acinetobacter(22%) and pseudomonas(16%).

Tables

Table- 1: Age and sex distribution of the study population.

AGE GROUP	MALE	FEMALE
< 1	2	4
1 to 15	0	0
16 to 30	6	1
31 to 45	8	3
46 to 60	11	6
> 60	41	18
TOTAL	68	32

Table- 2: XDR Gram negative bacilli isolated in various clinical samples.

Sample	Number	Acinetobacter	Klebsiella	Pseudomonas
Respiratory sample	36	25	6	5
Pus/ other fluid	28	17	7	4
Urine	27	5	18	4
Blood	9	7	2	0
Total	100	54	33	13

Table- 3: Sensitivity pattern of XDR Gram negative bacilli to old antibiotics

Antibiotics	Acinetobacter (54)	Klebsiella (33)	Pseudomonas (13)
Minocycline	42(78%)	7(21%)	0
Fosfomycine	51(94%)	33(100%)	10(77%)
Chloramphenicol	12(22%)	11(33%)	2(16%)
Colistin	52(98%)	33(100%)	12(92%)

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