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Brief Overview about *Enterobacterales* Antibiotic Resistance

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Abstract: Enterobacterales, a family of Gram-negative bacteria inhabiting the human gut and environment, pose a significant threat to global health due to their escalating antibiotic resistance. This resistance, driven by a complex interplay of factors, renders infections caused by these bacteria increasingly difficult, if not impossible, to treat with conventional antibiotics. The mechanisms underlying this resistance are diverse and include the production of extended-spectrum β-lactamases (ESBLs), carbapenemases, and aminoglycoside-modifying enzymes, which inactivate or reduce the effectiveness of various antibiotic classes. Horizontal gene transfer, facilitated by plasmids and transposons, plays a crucial role in the rapid dissemination of resistance genes across diverse Enterobacterales species, accelerating the emergence of multidrug-resistant (MDR) and pan-drug-resistant (PDR) strains. The clinical impact of Enterobacterales antibiotic resistance is profound, leading to increased morbidity, mortality, prolonged hospital stays, and escalating healthcare costs. Infections caused by resistant Enterobacterales, such as urinary tract infections, bloodstream infections, pneumonia, and intra-abdominal infections, are associated with higher treatment failure rates and worse patient outcomes. The rise of carbapenem-resistant Enterobacterales (CRE) is particularly alarming, as carbapenems represent a last-line defense against severe infections. CRE infections often necessitate prolonged hospitalization, the use of toxic alternative therapies, and are associated with significantly increased mortality. Contributing factors to the rise in resistance include the widespread use and misuse of antibiotics in human and veterinary medicine, coupled with inadequate infection control practices in healthcare settings. The selective pressure exerted by antibiotics drives the selection and amplification of resistant strains, while poor sanitation and hygiene practices contribute to the dissemination of resistant bacteria in the community. Addressing this escalating crisis necessitates a multi-pronged approach encompassing prudent antibiotic stewardship programs, improved infection control measures, the development of novel diagnostic tools for rapid detection of resistance, and the exploration and development of new antibiotics and alternative therapeutic strategies such as phage therapy and immunotherapies. Failure to implement comprehensive strategies will result in a future where common infections become untreatable, jeopardizing global health security.

Keywords: *ENTEROBACTERALES, Antibiotic Resistance*

Introduction.

Enterobacterales are Gram-negative, non-spore-forming, facultative anaerobic rod-shaped bacteria belonging to the Pseudomonadota phylum and Gammaproteobacteria class. This order includes seven validly published families, encompassing species such as *Escherichia coli*, *Klebsiella*, *Yersinia pestis*, *Shigella*, *Salmonella*, *Proteus*, *Serratia*, and *Citrobacter*, among other enteric pathogens. These bacteria constitute a small portion of the healthy human gut microbiota; however, disruption of this commensal relationship can lead to pathogen-related infections. [1]

Enterobacterales possess characteristic phenotypic traits, including a Gram-negative stain reaction, a negative oxidase test, a positive catalase test, acid production from glucose fermentation, and nitrate reduction, although exceptions exist. They cause a wide range of clinical diseases, including foodborne enteritis and zoonotic infections. [2]

Many Enterobacterales species are pathogenic to humans and livestock, such as *Salmonella* spp., pathogenic *Escherichia coli*, *Yersinia enterocolitica*, *Shigella* spp., and *Cronobacter* spp. Conversely, numerous members are considered opportunistic pathogens, including *Klebsiella* spp., *Serratia* spp., *Proteus*, *Hafnia* spp., and *Citrobacter* spp. The gut is a primary source of these potential pathogens, contributing to various infections such as implant-associated infections, meningitis, brain abscesses, biofilm-related infections, bacterial prostatitis, catheter-associated urinary tract infections, and nosocomial pneumonias, which can lead to sepsis or death. [3]

Escherichia coli (*E. coli*) is a well-studied bacterium colonizing the human gut shortly after birth. It comprises hundreds of identified strains, encompassing both commensal and pathogenic clones that cause infections in the gut and other tissues. Eight *E. coli* pathotypes have been identified, including six intestinal pathotypes and two extraintestinal pathogenic *E. coli* (ExPEC). *E. coli* is included in the WHO Bacterial Priority Pathogens List, highlighting its global impact concerning burden, transmissibility, treatability, and prevention.

Extraintestinal pathogenic *E. coli* (ExPEC) strains, including uropathogenic *E. coli* (UPEC) and meningitis-associated *E. coli* (NMEC), are highly versatile and responsible for numerous human infections in healthcare and community settings. [4]

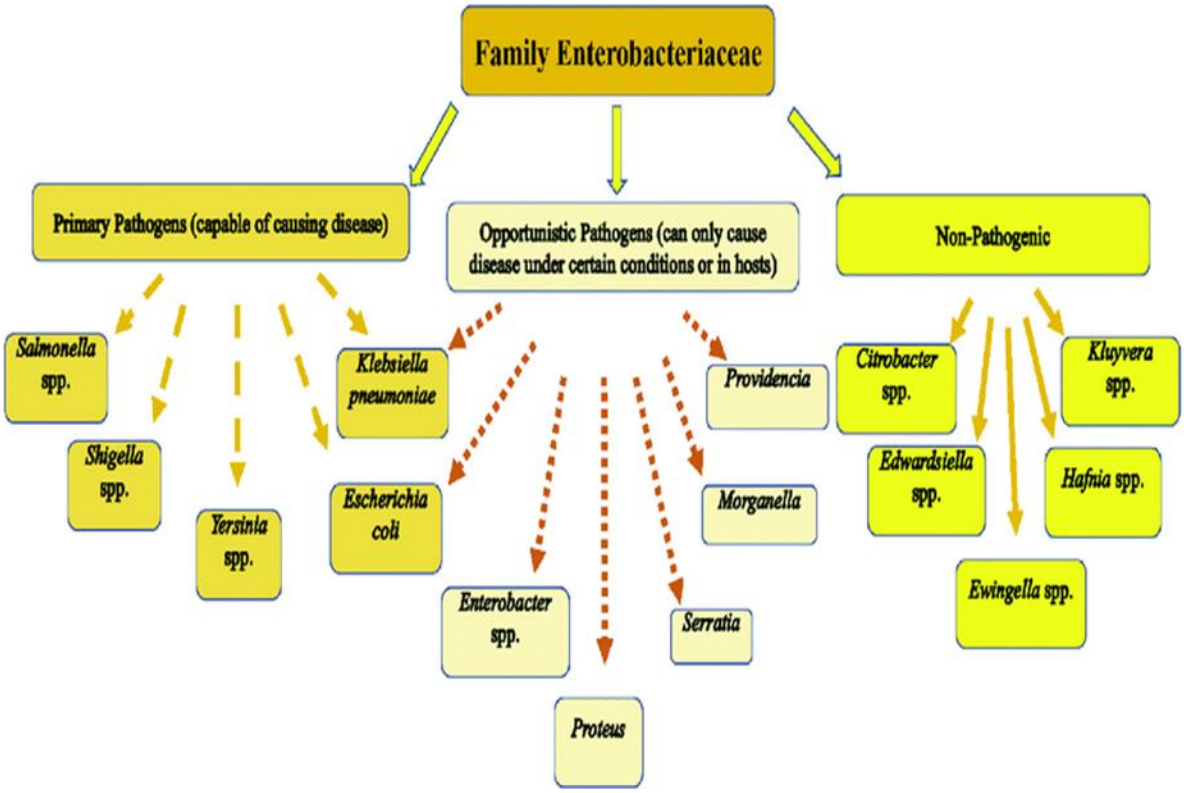
Citrobacter, *Enterobacter*, and *Serratia* genera species (CES) are non-spore-forming bacilli widely distributed in the environment and the digestive tract of animals and humans. They share similar biochemical characteristics and prevalence. Recently, they have emerged as significant pathogens in UTIs, both in community and nosocomial settings, frequently causing outbreaks, especially in intensive care units. CES bacteria are part of the "SPICE" group (Serratia, Pseudomonas, indole-positive Proteus, Citrobacter, and Enterobacter) and acquire numerous resistance mechanisms, complicating their management. They possess intrinsic penicillinases and AmpC-β-lactamases, conferring resistance to penicillins, several β-lactam/β-lactamase combinations, first- and second-generation cephalosporins, and cefoxitin. Furthermore, *Serratia* species exhibit high resistance to doxycycline, nitrofurantoin, colistin, and most aminoglycosides. [5]

Klebsiella is a Gram-negative, encapsulated pathogen causing numerous nosocomial infections, particularly pneumonia, urinary tract infections, and wound infections. *Klebsiella pneumoniae* (*K. pneumoniae*) is the most significant species, followed by *K. oxytoca*. However, it can also cause severe community-acquired infections, including endophthalmitis, pneumonia, necrotizing fasciitis, non-hepatic abscesses, and meningitis. The polysaccharide capsule is a crucial virulence factor enabling evasion of opsonization and phagocytosis. Globally, *K. pneumoniae* accounts for approximately 11.8% of healthcare-associated pneumonia, and nearly 50% of patients with pneumonia caused by carbapenem-resistant *K. pneumoniae* (CRKP) isolates die. [6]

The genus *Proteus* comprises four species: *Proteus mirabilis* (*P. mirabilis*), *P. vulgaris*, *P. penneri*, and *P. myxofaciens*. Closely related to *Morganella* and *Providencia* spp., *P. mirabilis*, a dimorphic Enterobacterales, accounts for approximately 40% of hospital-acquired infections and is a major cause of catheterized urinary tract infections in immunocompromised individuals, often complicated by urolithiasis and permanent renal damage. The primary virulence factors of *Proteus* are swarming motility and urease activity. [7]

Salmonella, *Shigella*, *Yersinia*, and *Campylobacter* spp. (SSYC) are termed "classic bacterial enteric pathogens," causing both community-acquired enterocolitis and critical hospital-acquired infections, such as septicemia. *Yersinia pestis*, the causative agent of bubonic plague, can be weaponized for bioterrorism purposes. *Shigella* is considered the third most common foodborne bacterial pathogen. [8]

There is a rise in foodborne outbreaks caused by consumable products exceeding traditional sources. These include basil (*Shigella*), bagged salad (*Y. enterocolitica*), cookie dough (*E. coli*), and peanut butter, jalapeno, and serrano peppers (*Salmonella*). [9]



Salmonella is a significant cause of foodborne infections and outbreaks in humans. The genus is divided into two categories: *S. enterica* and *S. bongori*. Humans are the primary reservoir for *S. typhi* and *S. paratyphi* serotypes, causing enteric fever, while *S. typhimurium* has a broad host range, causing disease in various animals. [9]

Shigella is the etiological agent of bacillary dysentery or shigellosis. It comprises four subgroups (*S. flexneri*, *S. sonnei*, *S. dysenteriae*, and *S. boydii*) with numerous serotypes. [10]

Campylobacter is the most prevalent cause of human foodborne gastroenteritis in developed countries and among the leading causes of diarrheal diseases globally. Infections are primarily caused by *Campylobacter jejuni* and *Campylobacter coli*. *Campylobacter* spp. are zoonotic pathogens, with various species isolated from human clinical samples. Several neuropathological disorders, including reactive arthritis, Guillain–Barré syndrome (GBS), and Miller Fisher syndrome (MFS), have been linked to *Campylobacter* infections. [11]

The WHO and CDC identify drug-resistant Enterobacterales as extremely concerning pathogens, particularly in ICUs. In US and European hospitals, multidrug resistance rates are higher in ICUs than non-ICU wards, involving

common Gram-negative organisms (*E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Acinetobacter* spp., and *Proteus mirabilis*). [12]

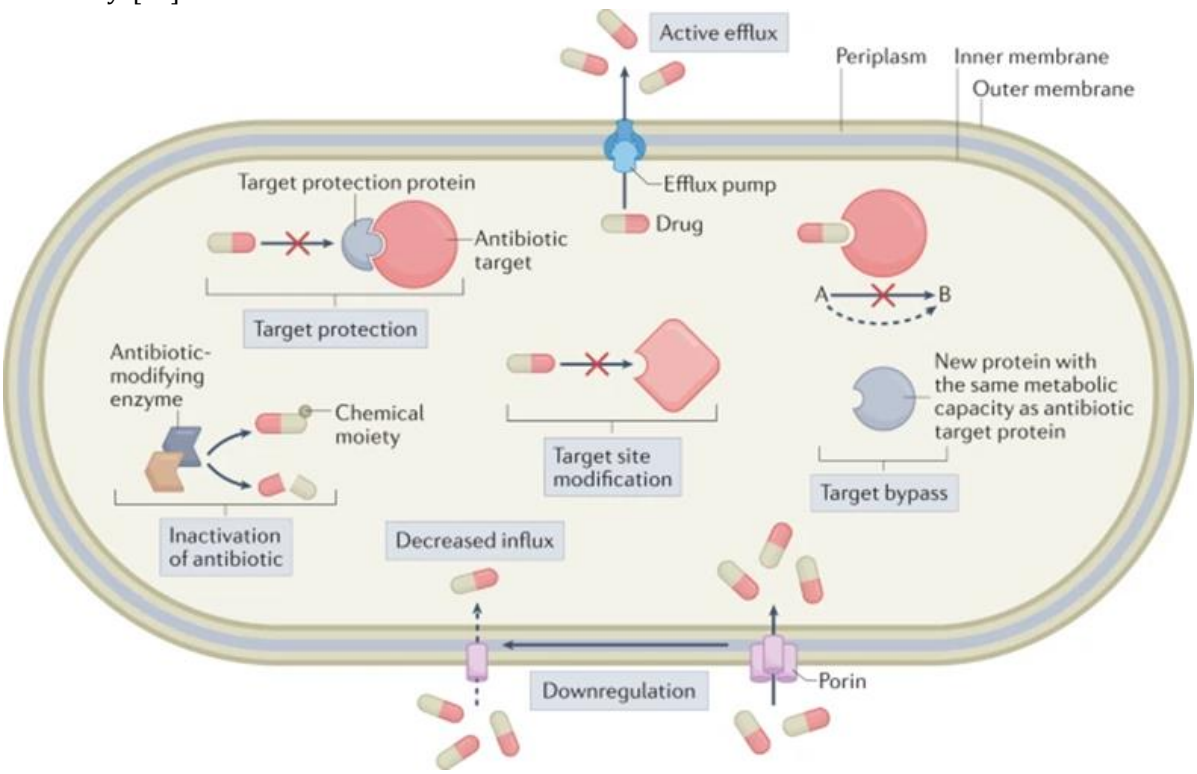
Microorganisms exhibiting antimicrobial resistance are categorized as multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR), with newer categories of difficulty-to-treat resistance (DTR) and modified DTR. MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories, XDR as non-susceptibility to at least one agent in all but two or fewer categories, and PDR as non-susceptibility to all agents in all categories. [13]

The costs and benefits of acquiring resistance are better understood, along with the role of plasmid-host-AMR gene interactions in the expansion of genes and strains. The carbapenemase KPC in *Klebsiella pneumoniae* sequence type 258 exemplifies the acquisition of specific resistance genes by globally dominant clones. [14]

Antibiotic resistance mechanisms include natural and acquired resistance. Acquired resistance mechanisms involve: (1) drug uptake limitation; (2) drug target modification; (3) drug inactivation; and (4) drug efflux. Mobile genetic elements (e.g., plasmids) are critical for spreading resistance genes. Gram-negative bacteria utilize all four mechanisms, whereas Gram-positive bacteria are less likely to use them due to limitations in drug uptake and efflux capacity. Intrinsic resistance can be innate or mediated. [15]

β-lactam antibiotics (penicillins, cephalosporins, carbapenems) and fluoroquinolones are frequently prescribed against Enterobacterales infections. Carbapenems are last-resort drugs for MDR Enterobacterales, but resistance to expanded-spectrum cephalosporins is increasing in ICU settings. [16]

Bacteria produce enzymes crucial for cell wall and nucleic acid biosynthesis, serving as antibiotic targets. β-lactamases hydrolyze β-lactam rings, rendering antibiotics ineffective. Gram-negative bacteria prevent antibiotic entry through the outer membrane via diffusion using protein channels (hydrophilic antibiotics) and a lipid-mediated pathway (hydrophobic antibiotics). Outer membrane modifications affect bacterial antibiotic sensitivity. [17]

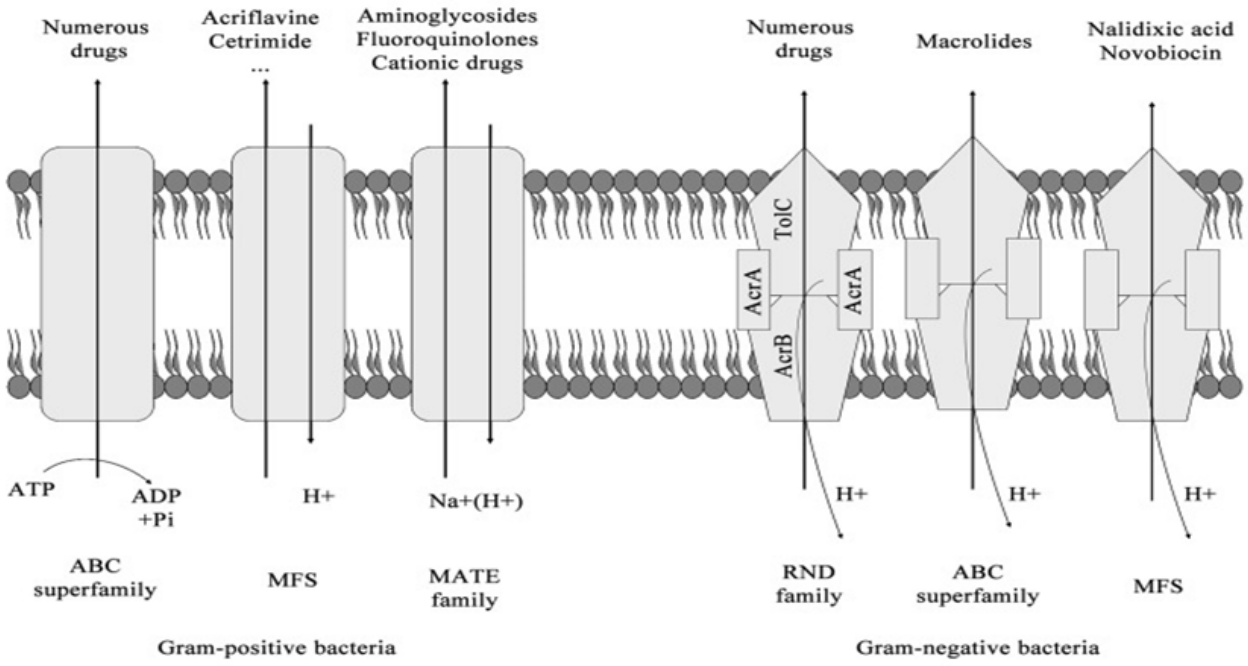


Six primary efflux pump families are classified based on structure, exported molecules, and energy supply. These pumps regulate the internal environment by eliminating toxic substances, including antimicrobial agents. The RND family is unique in not being a singular pump. Tet efflux pumps (MFS family) use proton exchange to extrude tetracyclines. Biofilm formation enhances antibiotic resistance by limiting drug influx and creating diverse metabolic states that promote bacterial survival and horizontal gene transfer. [18]

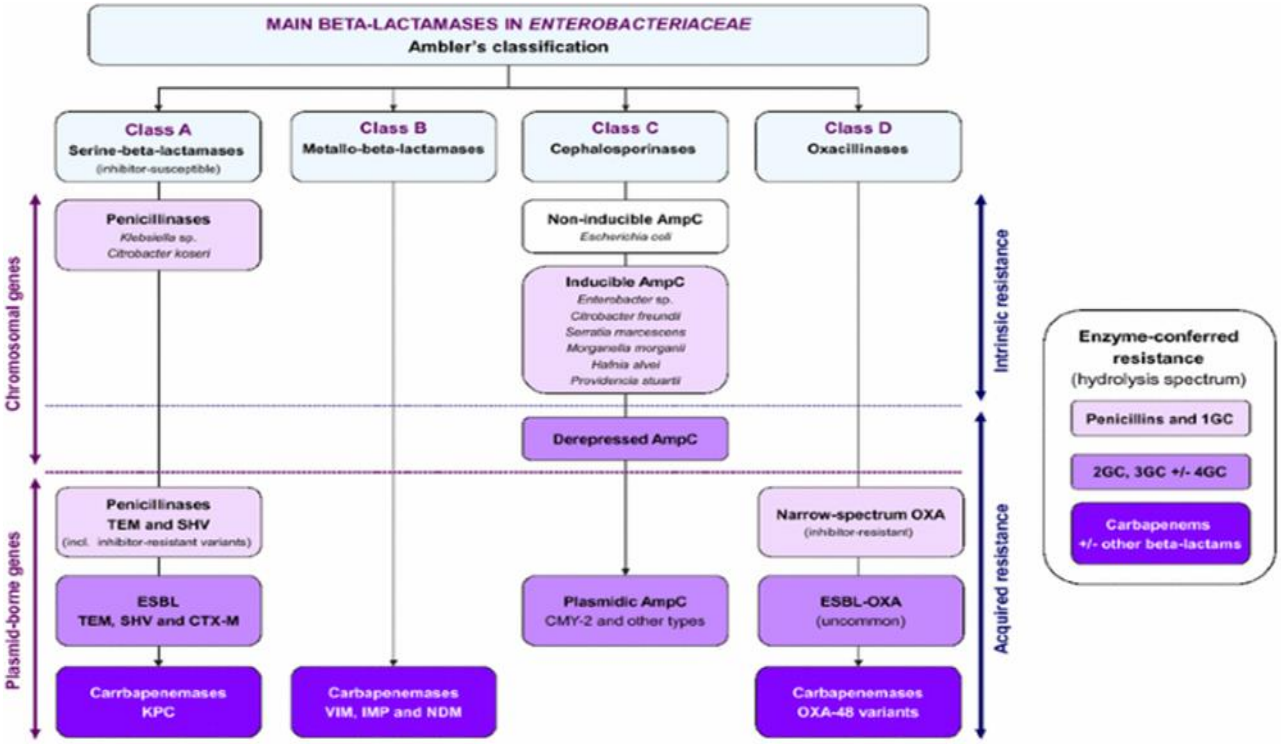
Aminoglycoside resistance involves aminoglycoside-modifying enzymes (AMEs) that hinder antibiotic activity. AME genes are transferred among bacteria through plasmids, transposons, integrons, and natural transformation or transduction. Intrinsic resistance exists in some species, while others acquire AME-encoding genes on plasmids. Other mechanisms include increased efflux, decreased permeability, and modifications of the 16S ribosomal A-site. New Delhi metallo-β-lactamases (NDM)-producing strains exhibit resistance to all aminoglycosides except neomycin. [19]

β-lactam resistance mechanisms involve hydrolysis by β-lactamases, alterations in outer membrane proteins (OMPs), penicillin-binding proteins, and increased efflux pump activity. Enterobacterales are known for extended-spectrum β-lactamases (ESBLs) production. ESBL-producing Enterobacterales are resistant to most β-lactams, often with co-resistance to fluoroquinolones, cotrimoxazole, and aminoglycosides. [20]

Tetracycline resistance involves the acquisition of *tet* genes related to efflux pumps, ribosomal protection, and enzymatic modifications. Several plasmid genes contribute to resistance, including *otr* and *tet* genes. *tet* genes encode membrane proteins that extrude tetracyclines or protect ribosomes. [21]



Polymyxin resistance is mainly due to chromosomal mutations modifying membrane lipopolysaccharides (LPSs), reducing electrostatic attraction between lipid A and the polymyxin molecule. Other mechanisms include lipid A alterations and efflux pumps. Cross-resistance between colistin and polymyxin B is observed. Plasmid-mediated colistin resistance (*mcr* genes) contributes to the rapid spread of resistance. [22]



Sulfonamide resistance often arises from the acquisition of dihydropteroate synthase (DHPS) genes in integrons, preventing drug inhibition. Three DHPS gene types (*sul1*, *sul2*, *sul3*) are known. [23]

AMR is projected to cause 10 million deaths annually by 2050, leading to increased morbidity and mortality and significant public health challenges. Biofilm formation enhances antibiotic resistance by creating a protective matrix, generating nutrient and oxygen gradients that support bacterial survival, and facilitating horizontal gene transfer. [23]

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