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Opinion Article

Blood System Dwelling Microbiota. Application of Colistin

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It is about "Septic shock" Defined as sepsis with hypotension requiring blood vessels Constriction agents treatment to keep a mean blood pressure of more than 65 mm Hg and a serum lactate level exceeding 2 mmol/L (18 mg/dL) after proper fluid resuscitation. Sepsis has a risk of *mortality* and long-term *morbidity*. Septicemia is a dangerous blood infection. Known as blood poisoning. It happens when a bacterial infection elsewhere in the body, enter the bloodstream. The danger because the bacteria and their secreted toxins [1a,b].The currently used diagnostics can rapidly identify pathogens in a patient's gut microbiome, researchers have found evidence that a patient's own microbiome may be the source of hospital-acquired bloodstream infections (BSIs).Sepsis is life-threatening [2a,b].

Abstract

Humans are now accepting to be in a complex symbiosis with a diverse ecosystem of microbial organisms, including bacteria, fungi, and viruses. Efforts to characterize the role of these microbes (the microbiome), in human health, Researchers describe and explore interactions between the microbiota and humans, with a focus on the microbiota's role in hematopoiesis and hematologic diseases [3]. Blood, in healthy organisms is regarded as a 'sterile' environment: it lacks microorganisms. Dormant or not-immediately-culturable forms are there, however, as intracellular dormancy is well documented. We point out here that a great many pathogens can survive in blood and inside erythrocytes. It is solved by improved culturing methods, and we ask how common this would be in blood. Several recent research have uncovered an authentic blood microbiome in several diseases. The source of these organisms is the gut (in particular when it shifts composition to a pathogenic state, known as 'dysbiosis'. We here use the term 'autopoiesis' for microbes that appear in places other than their regular location. Autopoiesis may be a factor to the dynamics of some of the inflammatory diseases. Overall, it seems that many more chronic, may be treatable using bactericidal antibiotics or vaccines [4].

How Do Gut Bacteria Escape Into Blood?

If the gut microbiome is seen as the source of the blood microbiome, it is necessary to establish which kinds of conditions might permit this in the absence of real physical damage (as may, for instance, be caused by surgery) leading to microbial translocation. Researchers mention three possible points of entrance for bacteria into the surrounding (sterile) tissues [5]: by dendritic cells via processes between epithelial cells, not affecting tight junction function, via injured/inflamed epithelium with dysfunctional epithelial barrier, and via M cells (M cells are specialized epithelial cells of the mucosa-associated lymphoid tissues.), overlying Peyer's patches (Peyer's patches are small masses of lymphatic tissue found throughout the ileum region of the small intestine.),as specialized cells providing access of microbial products to antigen presenting cells. We discuss bacterial translocation in this context in the following sections. One of the common drugs used nowadays in cases of sepsis is colistin. Here is some on Colistin.

Using Colistin

The explosive emergence of bacterial infections that are incurable, demands a need for new antimicrobial treatments and new drugs. Thousands die in healthcare facilities due to this situation – antibiotic-resistant microbial infections. Cyclic antimicrobial peptides – the quest for antimicrobial agent to combat resistant microbes recent years research afforded some peptide-based agents that are the very potent agents in the combat against resistant microbes. Research based on such compounds, may give rise to a new trend in the quest of remedy to the vicious killers that transform modern medicine to a better treatment of Hospital Acquired Infection (HAI), or the nosocomial pandemic. Statistics teach that sepsis kills many people

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every year. In the United States, patients hospitalized for septicemia are 8 times more susceptible to mortality than those suffering other infections, with over 200,000 deaths in 2008.[6] Statistics show that over one-third of patients with sepsis treated in an intensive care unit die in the hospital.[7] Bacterial sepsis is the most expensive condition treated in U.S. hospitals, costing more than $20 billion in 2011.Sepsis is usually caused by Gram-positive (G+ve) Staphylococcus Aureus and Gram-negative (G-ve) Klebsiella spp., Escherichia coli, and Pseudomonas aeruginosa. There has been a frightening rise in highly drug-resistant (G-ve) bacteria that are able to overcome almost every known antibiotic, The current clinical antimicrobial pipeline is mainly populated by G+ve drug candidates [8a-c] .The upper box depicts the structures of the "Veteran" but highly bioactive (Gramicidin [9], 1940, Polymyxins 1947) that are able to eradicate the most aggressive

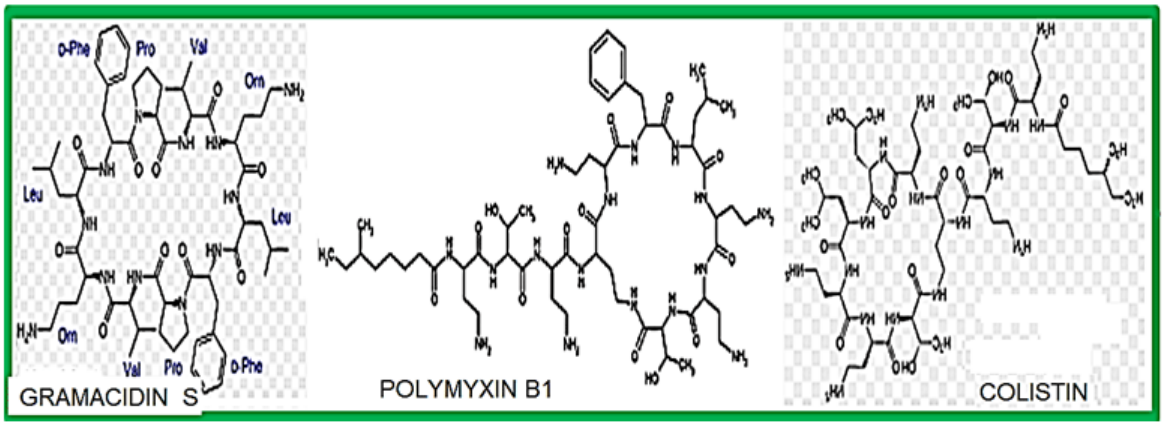

Figure 01: Highly active cyclic polypeptide agents from the 1940s

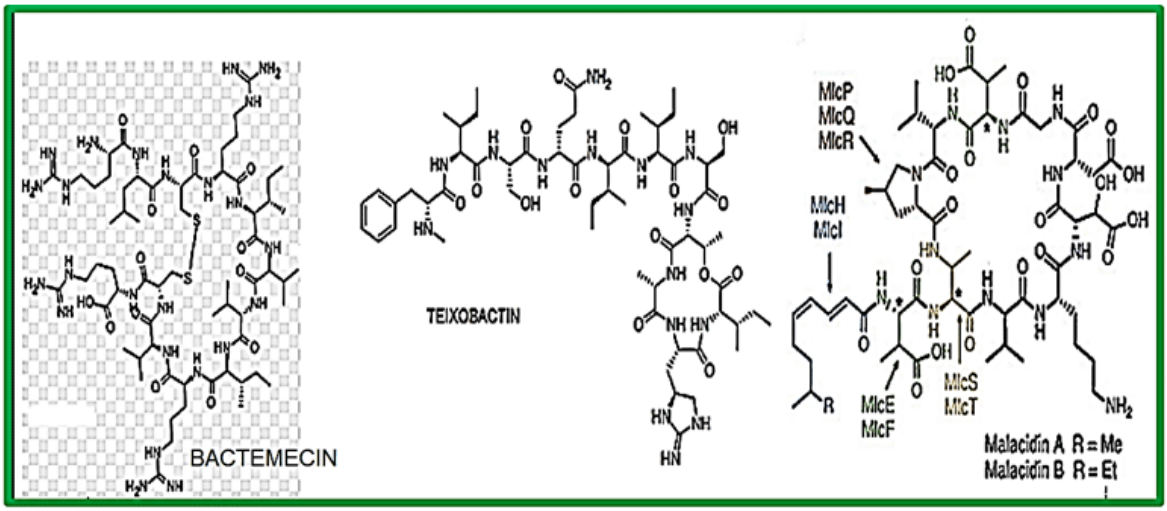

Figure 02: Recently discovered antimicrobial agent based on cyclic polypeptides from the 2000's

resistant bacteria strands, However, these are highly toxic to humans. Cyclic peptides are leading the current anti-resistant antimicrobials. Bactenecin, Teixobactin [10], and the Milacainides [11] are the most active that eradicate resistant bacteria strands. The finding new antibiotics to treat gram-positive infections like MRSA was good news but could not address the most pressing need. Our concern is the so-called gram-negative bacteria which are difficult to treat and where resistance is on the increase.

The History of Colistin in Short

Historically, colistin was one of the first antibiotics to be developed in the early 1950s [12], but it wasn't used to treat hard infections in human medicine because it is very toxic and using it is involved with damaging effects. In general, antibiotics have side effects which affect different individuals to different extents, but few of them are as violent as those

caused by colistin, which can cause nephrological and neurological damage that is occasionally irreversible. It's not surprising, therefore, that pharmaceutical companies assumed there would be no problem if colistin were aimed for use in livestock production, as products like Coliscour and Colibird. One of its uses has simply been to prevent or treat diarrhea in piglets that is caused by E. coli bacteria which spread very quickly on intensive farms and especially in piglets weaned at 3-4 weeks old because their immune systems do not function fully until they are about eight weeks of age. Colistin is currently applied for the treating of the Gram -veE. Coli and *salmonella* infections in veal calves and poultry. The statistic is that E. coli sepsis infections affect about 40,000 people a year in the UK, and every year kill about 7,000 patients including from time to time neonates which become infected in the birth canal [13]. "In March 2016(credit New York times. credit ref. [14]), the CDC reported that "superbugs" – bacteria that are highly resistant to antibiotics – are responsible for one out of seven infections caught in general hospitals, causing an annual estimate of 80,000 in the U.S. Several types of bacterial infections have evolved to 'deadly invader' status, and it's partially our fault. Among the 18 superbugs identified by the CDC are salmonella, drug-resistant tuberculosis, and a staph bacterium known as MRSA (Methicillin-resistant Staphylococcus aureus). These bugs are gaining 'super' status because we've been careless and stupid with antibiotics like Colistin, rendering them useless, if not dangerous. In other words, we're helping create deadly types of bacteria. The revival of Colistin is the rehabilitation of a renal [15] damaging agent; it is the best drug to apply when there is no other choice". Chickens raised in India for food have been dosed with some of the strongest antibiotics known to medicine, in practices that could have repercussions throughout the world. Colistin is one of only four antibiotics which can be added to the drinking water of egg-laying hens suffering where there is no requirement to observe any withdrawal period at all [16] can be slaughtered for human consumption just 24 hours after being treated with colistin. Colistin is a complex of two polypeptide antibiotics, the drug is composed of colistin A and B. Nowadays, colistin is increasingly put forward as "last resort" or first-line treatment for severe multidrug-resistant, Gram-negative bacterial infections, particularly in the intensive care setting. It is frequently applied is cases of septicemia. Polymyxins are polypeptide antibiotics, with a primary effect of membrane-damaging due to their selective binding to the lipopolysaccharide of Gram-negative bacteria [17]. Their nephew- and neurotoxic side effects [18] limited their use, however, in the last decade the emergence of multidrug-resistant Gram-negative bacteria led to the reintroduction of polymyxins into clinical practice. The emergence of multiple-drug-resistant (MDR) gram-negative microorganisms, including *Pseudomonas aeruginosa* and *Acinetobacter baumannii,* is a major concern worldwide.[19] MDR *A. baumannii* and *P. aeruginosa* are important causes of nosocomial infection, and outbreaks of these

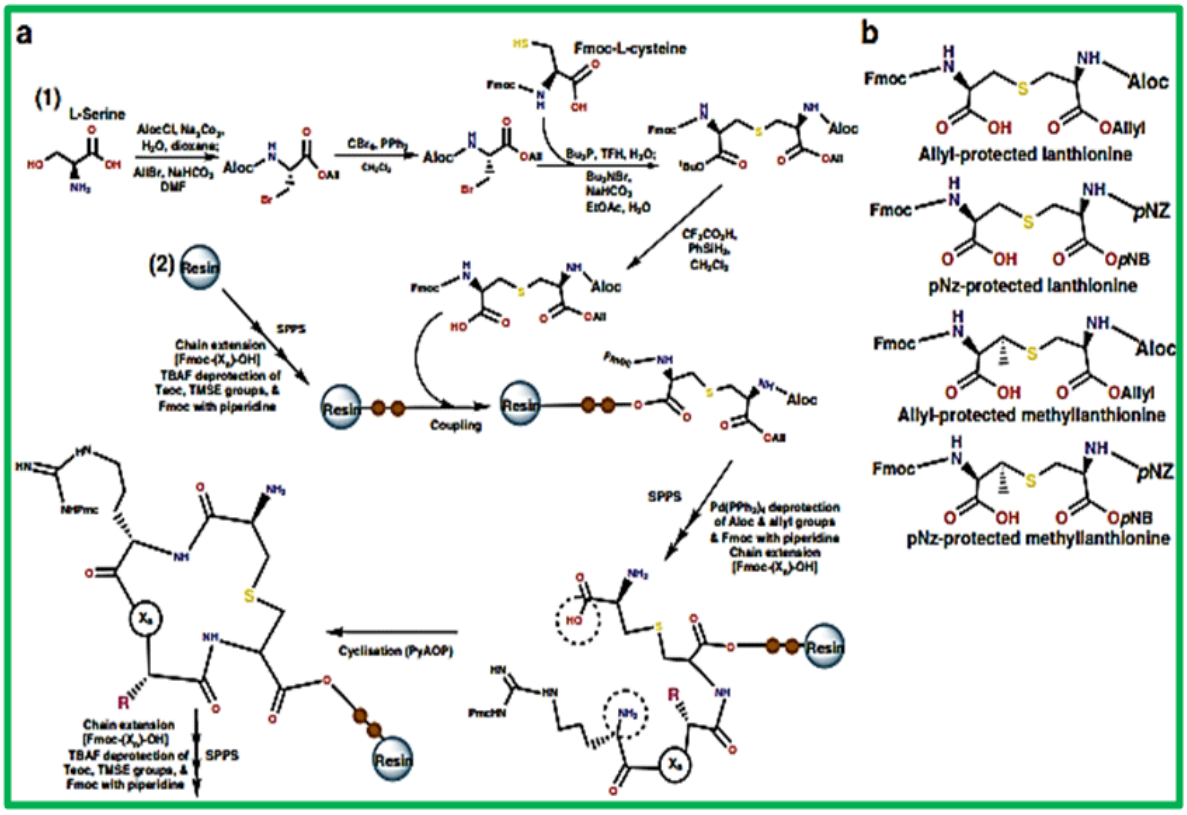

Figure 03: The simplified scheme, exemplifying chemical synthesis of lanthionine ring using OPLs. (Credit ref. [ ]).

microorganisms, which are resistant to most available antimicrobial agents, have been reported in burn units, intensive care units (ICUs), cancer centers, and patients with cystic fibrosis [20. The WHO recognized the importance of colistin in anti-microbial therapy. Expert Committee on Biological Standardization (1964) decided that there was a need for an international reference preparation of colistin methane sulfonate and requested the National Institute for Medical Research, London, to obtain a suitable sample and make a preliminary evaluation of it [21].

The mechanism [22] for the antibiotic activities of the polymyxins and octapeptins has been elucidated by research using a broad range of experimental techniques. Since 1947, when polymyxin was first isolated, there have been tremendous advances in our knowledge of membrane structure. It is these techniques which will provide a detailed molecular mechanism for the effects of these peptide antibiotics on membrane structure. Also, the large number of antibiotic derivatives available should be exploited more extensively for structure-function correlations. The ultimate goal is to correlate the biological properties of these peptides with their effects on the physical properties of membranes and to rationalize these events regarding lipid-peptide interactions.

The chemical synthesis was published,and a patent application was filed.

Increasing number of infection cases caused by multi resistant Gram-negative bacteria or multidrug-resistant organism (MDRO) has become a major problem worldwide since there has been a lot of resistance to many classes of antibiotics. *Colistin* [23] or polymyxin E is an old antibiotic, which has been used since 1959 for treating infection caused by Gram-negative MDRO. It was revealed that colistin has side effects of nephrotoxicity and neurotoxicity; therefore, the use of this antibiotic was stoppedand it was replaced by other antibiotics which were effective and were considered safer at that time.

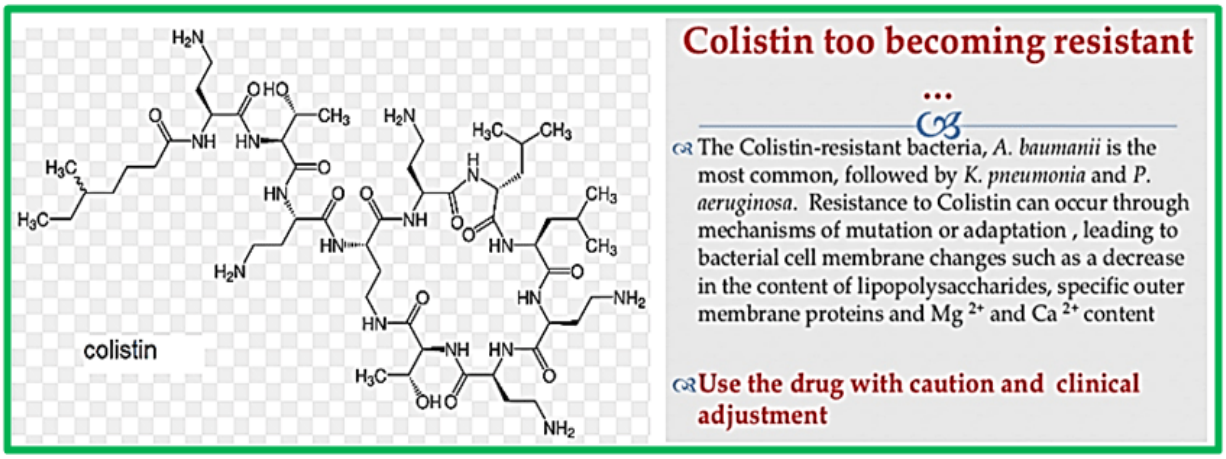

Figure 04:

There is a growing number of cases of infections caused by multi-resistant Gram-negative (MDRO) against the available antibiotics. There is only a little availability of antimicrobial alternative agents; therefore, scientists, in their quest for remedy, are investigating back to old options, which have been proven to be effective against multi-resistant Gram-negative bacteria, the old antimicrobial that has been long forgotten, i.e., colistin, as an alternative treatment against Gram-negative MDRO. Here are some facts on Colistin: Colistin is a real-life saver, It is one of the most active agents in the combat with the Gram-negative resistant bacteria. Its mechanism of action disrupts both the inner and outer membranes of the Gram-negative bacteria.

Polymyxins are antibiotics, structure consisting of a cyclic peptide with a long hydrophobic tail. They disassemble the structure of the bacterial cell membrane by interacting with its phospholipids. They are produced by the Gram-positive bacterium Bacillus polymyxin and are selectively toxic for Gram-negative bacteria due to their specificity for the lipopolysaccharide molecule that exists within many Gram-negative outer membranes. Colistin – A Polymyxin E Colistin is a cationic polypeptide antibiotic from the polymyxin family that was first introduced in 1962 but abandoned in the early 1970s because of initial reports of severe toxicities. However, a recent increase in the prevalence of multidrug-resistant (MDR) Pseudomonas aeruginosa and the lack of novel agents in development calls for a need to re-examine the role of colistin therapy in patients with cystic fibrosis. What is Colistin? - Colistin (also called polymyxin E) belongs to the polymyxin group of cyclic peptides antibiotics. It was first isolated in Japan in 1949 from Bacillus polymyxin var. colistin and became available for clinical use in 1959. Colistin was given as an intramuscular injection for the treatment of gram-negative infections but fell out of favor after amino glycosides became available because of its significant side effects. It was later used as topical therapy as

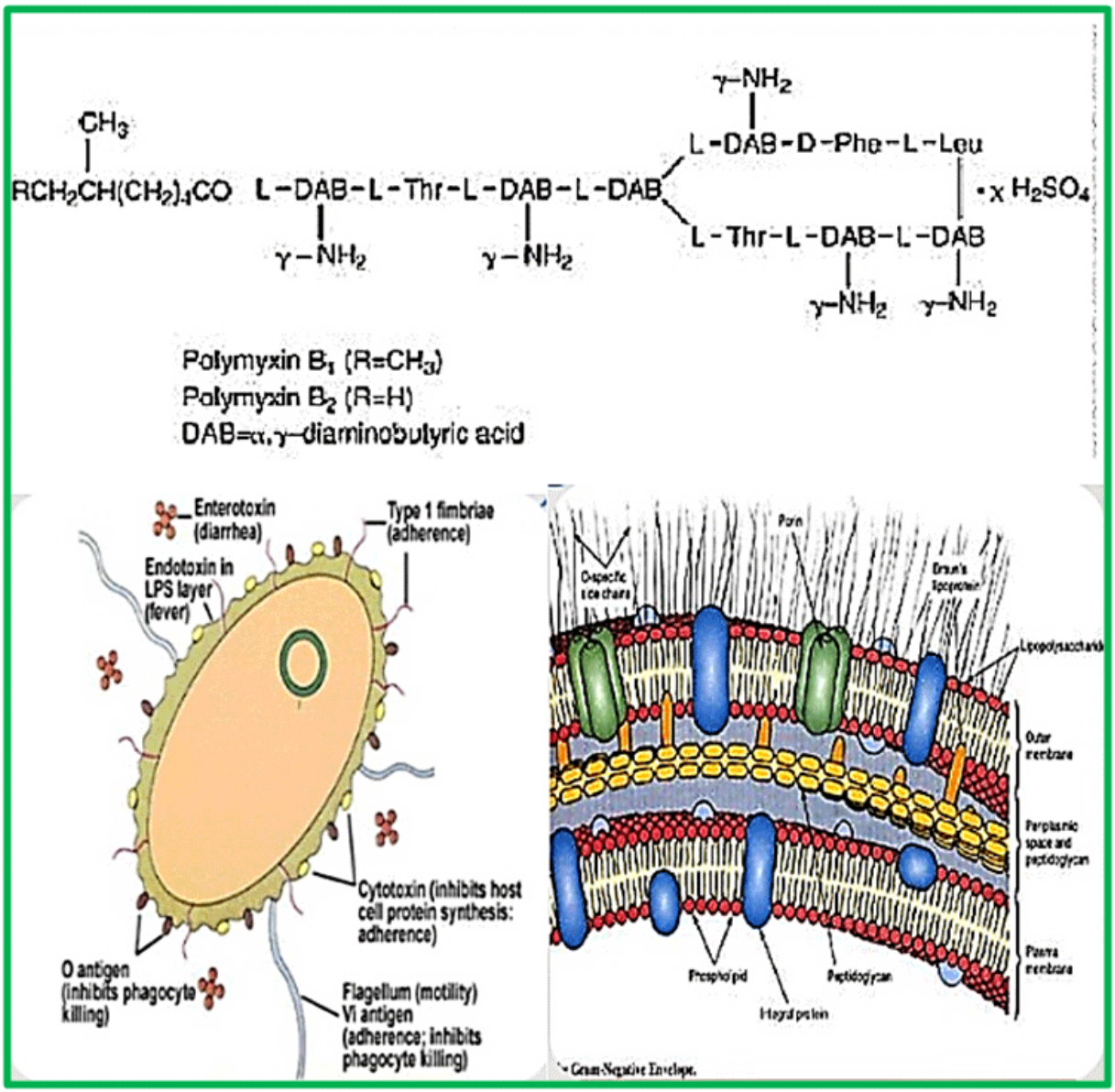

Figure 05:

part of selective digestive tract decontamination and is still used in an aerosolized form for patients with cystic fibrosis.

Structure of Polymyxins: Polymyxin B Sulfate is one of a group of basic polypeptide antibiotics derived from B polymyxin (B aero porous). Polymyxin B sulfate is the sulfate salt of Polymyxins B1 and B2, which are produced by the growth of Bacillus polymyxin.

Colistin: The target of the antimicrobial activity of colistin is the bacterial cell membrane Colistin also has potent anti-endotoxin activity. The endotoxin of G-N bacteria is the lipid A portion of LPS molecules, and colistin binds and neutralizes LPS 7

Mechanism of Action

Bactericidal. Bind to lipopolysaccharides (LPS) & phospholipids in the outer cell membrane of G(-) bacteria, neutralize LPS & prevent pathophysiologic effects of endotoxin. Resistance is uncommon. Disk diffusion method cannot be used

The spectrum of Activity: *Pseudomonas & A. baumanniiE. Coli, Enterobacter H. influenza, Bordetella pertussis. Legionella, Klebsiella spp. Salmonella spp., Shigella spp. Stenotrophomonas maltophilia* [25]

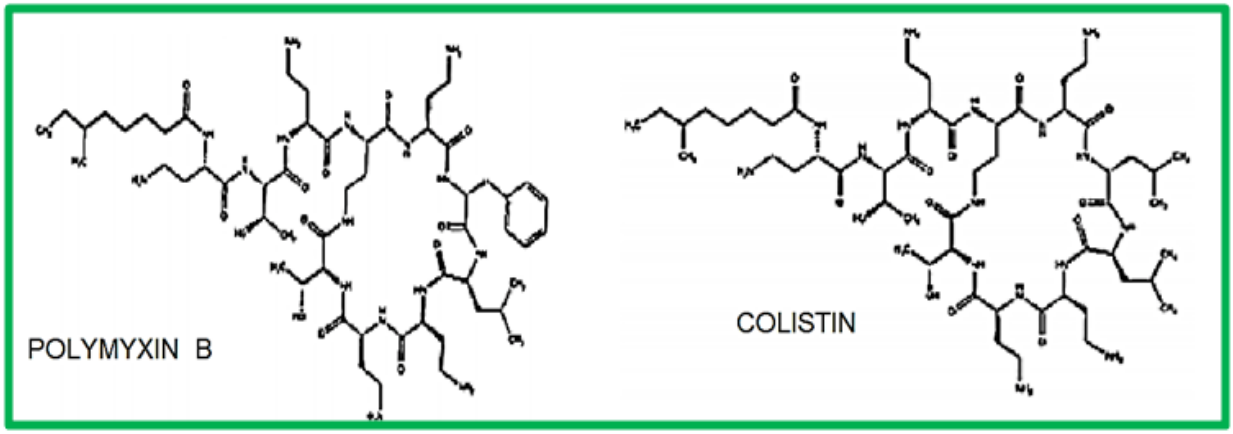

Figure 06:

The use of colistin [26]

Due to the major concerns for neuro- and nephrotoxicity [27], parenteral use of polymyxins has until recently been limited and polymyxins were mainly used for ophthalmic and topical infections [28]. Cystic fibrosis patients have been an exception to this practice for decades, and such patients have received systemic or nebulized colistin to control lower airway bacterial infections and their complications [29]. During the last five years, two major indications have renewed the interest for polymyxins in human medicine, namely as part of surgical prophylaxis via SDD and for the treatment of MDR Gram-negative healthcare-associated infections. For human patients, two salt forms of polymyxin E (colistin) have been widely commercially available, namely colistin sulfate and colistimethate sodium (CMS, syn colistin methanesulphonate, colistin sulphonyl methane, pentasodiumcolistimethanesulphate). CMS is a prodrug of colistin and is microbiologically inactive [30]. It is administered predominantly as parenteral formulations and via nebulization. After administration, CMS is hydrolyzed to colistin, which is the base component that is responsible for its antibacterial activity. Colistin sulfate is available in tablets and syrup for SDD and as topical preparations for skin infections. CMS is available for administration intravenously, intramuscularly as well as topically via aerosol (nebulization) or intraventricular administration. Total consumption (reflecting topical, inhalational and systemic routes of administration combined) varied widely between EU/EEA countries and doubled between 2010 and 2014 [31] following the rise in MDR Gram-negative pathogens involved in healthcare-associated infections.

Veterinary medicine

Within the EU MSs, colistin and polymyxin B are authorized nationally. The main indication for colistin in veterinary medicine is an infection of the gastrointestinal tract caused by non-invasive E. coli in pigs, poultry, cattle, sheep, goats, and rabbits. Colistin is also used in laying hens and cattle, sheep and goats producing milk for human consumption. Colistin is also active against endotoxins produced by some E. coli strains in the gastrointestinal tract. Typically, colistin products are administered orally, in feed, in drinking water, as a drench, or through milk replacer diets. [32]. Colistin and polymyxin B have been approved for topical administration, as in human medicine [33,34], consumption data are not available separately for this food production sector. In the Danish monitoring program (DANMAP), details on consumption do not refer to the use of colistin in fish. Recent reports indicate that bacteria developed resistance even towards colistin; Chinese scientists report on "Emergence of Colistin Resistance Gene *mcr-8* and Its Variant in *Raoultellaornithinolytica*", (credit ref. [35])

References

1.

a. Haak BW, Prescott HC[2], Wiersinga WJ (2018) "Therapeutic Potential of the Gut Microbiota in the Prevention and treatment of Sepsis"; Front Immunol 9: 2042.

b. Cabrera-Perez J, Badovinac VP, Griffith TS, (2017) " Enteric immunity, the gut microbiome, and sepsis: Rethinking the germ theory of disease"; Exp Biol Med (Maywood) 242(2): 127–139.

2.

a. Ricki Lewis, (2018) "Gut Microbiome May Seed Sepsis"; Medscape.

b. Dethlefsena L, Relmana DA (2011)" Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation", PNAS 108(Suppl 1): 4554-4561.

3. Manzo VE, Bhatt AS (2015) "The human microbiome in hematopoiesis and hematologic disorders"; Blood 126(3): 311-318.

4. Potgieter M, Bester J, Kell DB, Pretorius E, (2015) "The dormant blood microbiome in chronic, inflammatory diseases"; FEMS Microbiology Reviews 39(4): 567–591.

5. Wiest R, Lawson M, Geuking M (2014) "Pathological bacterial translocation in liver cirrhosis". J Hepatol 60(1): 197–209.

6. Lagu T, Rothberg MB, Shieh MS, Pekow PS, Steingrub JS, et al (2012) Hospitalizations, costs, and outcomes of severe sepsis in the United States 2003 to 2007. Crit. Care Med 40(3): 754–761.

7. Vincent JL, Marshall JC, Namendys-Silva SA, François B, Martin-Loeches I, et al (2014) Assessment of the world wide burden of critical illness: the intensive care over nations (ICON) audit. Lancet Respir Med 2(5): 380–366.

8

a. Torio CM, Andrews RM (2011) National Inpatient Hospital Costs: The Most Expensive Conditions by Payer, 2011: Statistical Brief #160. In Healthcare Cost and Utilization Project (HCUP) Statistical Briefs, Rockville (MD) Agency for Healthcare Research and Quality (US); 2006-2013 Aug.

b. Opal SM, Garber GE, LaRosa SP, Maki DG, Freebairn RC, et al (2003) Systemic host treatment effects of drotrecogin alfa (activated). Clin. Infect. Dis 37(1): 50–58.

c. Butler MS, Blaskovich MA, Cooper MA (2013) Antibiotics in theclinical pipeline in 2013. J. Antibiot 66(10): 571–591.

9

a. Burkhart BM, Gassman RM, Langs DA, Pangborn WA, Duax WL, et al (1999) " Gramicidin D conformation, dynamics and membrane ion transport"; Biopolymers 51(2): 129-44.

b. Benedict Rg, Langlykke Af (1947) "Antibiotic activity of Bacillus polymyxin"; J Bacteriol 54(1):

c. Landy M, Rosenman SB,Warren GH (1947) An antibiotic from Bacillus subtilis active against pathogenic fungi"; Journal of Bacteriology 54(1): 24

10. Chuchu Guo , Mandalapu D, Xinjian Ji, Jiangtao Gao, Zhang QI (2018) "Chemistry and Biology of Teixobactin"; Chem Eur J 24(21): 5406-5422

11 https://www.pharmazeutische-zeitung.de/index.php?id=74276

12. Gallardo-Godoy A, Muldoon C, Becker B, Elliott AG, Lash LH, et al (2016) " Activity and Predicted Nephrotoxicity of Synthetic Antibiotics Based on Polymyxin B"; J. Med. Chem 59(3): 1068−1077.

13. Richard Young (2015)" Resistance to colistin and what it means for farming".

14. Carroll AE (2016) "We're losing the Race against Antibiotic Resistance, but there's Also Reason for Hope"; The New Health Care.

15. 2018 "Warning over wider global health impacts after findings reveal hundreds of tonnes of colistin –the 'antibiotic of last resort' – are being shipped to India's farms".

16. Tom Batchelor (2018)" Farmers using powerful antibiotic 'of last resort' on healthy chickens to boost weight, report finds".

17. Kadar B, Kocsis B, Nagy K, Szabo D (2013) " The renaissance of polymyxins"; Curr Med Chem.; 20(30): 3759-73.

18. Spapen H, Jacobs R, Gorp VV, Troubleyn J, Honoré PM (2011)" Renal and neurological side effects of colistin in critically ill patients""; Annals of Intensive Care 1:14.

19.

a. Hanberger H, Diekema D, Fluit A et al (2001) Surveillance of antibiotic resistance in European ICUs. J Hosp Infect 48(3): 161-76.

b. Waterer GW, Wunderink RG (2001)Increasing threat of gram-negative bacteria. Crit Care Med 29(4): 75-81.

c. Jones RN (2001) Resistance patterns among nosocomial pathogens: trends over the past few years. Chest 119(2 suppl): 397S-404S.

d. Bergogne-Berezin E, Towner KJ (1996) Acinetobacter spp. nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin Microbiol Rev 9(2): 148-65.

e. Bergogne-Berezin E (2001) The increasing role of Acinetobacter species as nosocomial pathogens. Curr Infect Dis Rep 3(5): 440-444.

f. Chastre J, Trouillet JL (2000) Problem pathogens (Pseudomonas aeruginosa and Acinetobacter) Semin Respir Infect 15(4): 287-98.

20.

a. Corbella X, Montero A, Pujol M, Domínguez MA, Ayats J, et al (2000) Emergence and rapid spread of carbapenem resistance during a large

and sustained hospital outbreak of multi resistant Acinetobacter baumannii. J Clin Microbiol 38(11): 4086-95.

b. Levin AS, Mendes CMF, Sinto SI et al (1996) An outbreak of multi resistant Acinetobacter baumannii in a university hospital in Sao Paulo, Brazil. Infect Control Hosp Epidemiol 17(6): 366-8.

c. Santucci SG, Gobara S, Santos CR Fontana C, Levin AS (2003) Infections in a burn intensive care unit: experience of seven years. J Hosp Infect 53(1): 6-13.

d. Garner JS, Jarvis WR, Emori TG Horan TC, Hughes JM (1988) CDC definitions for nosocomial infections, 1988. Am J Infect Control 16(3): 128-40.

e. Cheng K, Smyth RL, Govan JR Doherty C, Winstanley C (1996) Spread of beta-lactam-resistant Pseudomonas aeruginosa in a cystic fibrosis clinic. Lancet 348(9028): 639-42.

21. Gillespie TA, Johnson PR, Notman AW Coia JE, Hanson MF (2000) Eradication of a resistant Pseudomonas aeruginosa strain after a cluster of infections in a hematology/oncology unit. Clin Microbiol Infect 6(3): 125-130.

22. Lightbown JW, Bond JM, Grab B (1973) "The international reference preparation of colistin methane sulfonate"; Bull World Health Organ 48(1): 75–80.

23. Storm DR, Rosenthal KS, Swanson PE (1977) "Polymyxin and related peptide antibiotics"; Annu Rev Biochem 46:723-63

24.

a. Ongey EL, Neubauer P (2016) " Lanthipeptides: chemical synthesis versus in vivo biosynthesis as tools for pharmaceutical production"; Microb Cell Fact 15: 97.

b. European Patent Specification, Ep 2 391 226 B1.

c. Craik DJ, Fairlie DP, Liras S, Price D (2013) The future of peptide-based drugs. Chem Biol Drug Des 81(1): 136–47.

25. Loho T, Dharmayanti A (2015) " Colistin: an antibiotic and its role in multi resistant Gram-negative infections"; Acta Med Indones 47(2):157-68.

26. 2012.Colistin in Multi Drug Resistant Bacteria.

27. EMA/CVMP/CHMP/231573/2016 Committee for Medicinal Products for Veterinary use (CVMP) Committee for Medicinal Products for Human Use (CHMP). "Updated advice on the use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health"

28. Koch-Weser J, Sidel VW, Federman EB, Kanarek P, Finer DC (1970) 'Adverse effects of sodium colistimethate. Manifestations and specific reaction rates during 317 courses of therapy', Annals of internal medicine 72(6): 857-868.

29. Falagas ME, Kasiakou SK (2005) 'Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections', Clinical infectious diseases: an official Publication of the Infectious Diseases Society of America 40(9): 1333-1341.

30.

a. Beringer P (2001) 'The clinical use of colistin in patients with cystic fibrosis', Current opinion in pulmonary medicine 7(6): 434-440.

b. Tappenden P, Harnan S, Uttley L, Mildred M, Carroll C (2013) 'Colistimethate sodium powder and to bramycin powder for inhalation for the treatment of chronic Pseudomonas aeruginosa lung infection in cystic fibrosis: systematic review and economic model', Health Technology Assessment 17(56): 1-181.

31

a. Bergen PJ, Li J, Rayner CR, Nation RL (2006) 'Colistin methanesulfonate is an inactive prodrug of colistin against Pseudomonas aeruginosa', Antimicrobial agents and chemotherapy 50(6): 1953-1958.

b. Falagas ME, Kasiakou SK (2005) 'Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections', Clinical infectious diseases: an official publication of the Infectious Diseases Society of America 40(9): 1333-1341.

c. Lim LM, Ly N, Anderson D, Yang JC, Macander L (2010) 'Resurgence of colistin: a review of resistance, toxicity, pharmacodynamics, and dosing', Pharmacotherapy 30(12): 1279-1291.

32

a. ECDC (2015) 'Summary of the latest data on antibiotic consumption in the European Union. ESAC-Net data.

b. Skov RL, Monnet DL (2016) 'Plasmid-mediated colistin resistance (mcr-1 gene): three months later, the story unfolds', Euro surveillance 21(9): 30155.

33.

a. Barton MH, Parviainen A, Norton N (2004) 'Polymyxin B protects horses against induced endotoxaemia in vivo', Equine veterinary journal 36(5): 397-401.

b. Moore, J.N., and M.H. Barton (2003) 'Treatment of endotoxemia', The Veterinary clinics of North America. Equine practice 19(3): 681-695.

c. Official Journal of the European Union. 2013. Commission Regulation (EU) No 122/2013 of 12 February 2013 amending Regulation (EC) No 1950/2006 establishing, in accordance with Directive 2001/82/EC of the European Parliament and of the Council on the Community code relating to veterinary medicinal products, a list of substances essential for the treatment of equidae 62: 291-307.

34. EMA/ESVAC (2015) 'European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption. Sales of veterinary antimicrobial agents in 26 EU/EEA countries in 2013 (EMA/387934/2015). Fifth ESVAC report. http://www.ema.europa.eu/docs/en_GB/document_library/Report/2015/10/WC500195687.pdf

35.

a. Xu Y, Tian X, Ren C, Huang H, Zhang X, et al (2012) "Analysis of colistin A and B in fishery products by ultra performance liquid chromatography with positive electrospray ionization tandem mass spectrometry', Journal of chromatography". B, Analytical technologies in the biomedical and life sciences 899: 14-20.

b. DANMAP, 2012. 'DANMAP 2011 –"Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark".

36. Wang X, Wang Y, Zhou Y, Wang Z, Wang Y, et al (2019) " Emergence of Colistin Resistance Gene mcr-8 and Its Variant in Raoultella ornithinolytica"; Front Microbiol 10: 228.