Medicinal Use of Lincosamides and Microbial Resistance to Them

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Abstract: Lincomycin and its derivatives are antibiotics exhibiting biological activity against bacteria, especially Gram-positive ones, and also protozoans. Lincomycin and its semi-synthetic chlorinated derivative clindamycin are widely used in clinical practice. Both antibiotics are bacteriostatic, inhibiting protein synthesis in sensitive bacteria, and they may even be bactericidal at higher concentrations that can still be reached *in vivo*. Clindamycin is usually more active than lincomycin in the treatment of bacterial infections, in particular those caused by anaerobic species; it can also be used for the treatment of important protozoal diseases, e.g. malaria, most effectively in combination with other antibiotic or nonantibiotic antimicrobials (primaquine, fosfidomycin, benzoyl peroxide). Resistance to lincomycin and clindamycin may be caused by methylation of 23S ribosomal RNA, modification of the antibiotics by specific enzymes or active efflux from the bacterial cell. In addition to the various medicinal applications and modes of microbial resistance to lincosamides, the review describes the chemical structures of lincosamide antibiotics and analytical procedures used for identification, separation and isolation of these compounds and their metabolites. The biosynthesis of lincomycin and related compounds and its genetic control are also briefly discussed.

Keywords: Lincomycin, clindamycin, lincosamide antibiotics, microbial resistance.

INTRODUCTION

Lincosamides form a small but important group of antibiotics with broad medicinal use produced by several *Streptomyces* species including *Streptomyces lincolnensis*, *S. espinosus*, *S. roseolus*, *S. pseudogriseolus*, *S. pseudogriseolus* var. *linmyceticus*, *S. celestis*, *S. variabilis* var. *liniabilis*, *S. vellosus*, as well as *Micromonospora halophytica* and *Actinomyces roseolus*.. Their chemical structure consists of an amino acid moiety (propylhygric acid) and a sugar moiety (methylthio-lincosamide). Natural and semisynthetic lincosamides are lincomycins A,B,C,D,S,K, celesticetins A,B,C,D, desalicetin, desalicetin D, and N-demethyl-celesticetin; the most important semi-synthetic derivative with high biological activity is the chlorinated derivative clindamycin. Clindamycin is active against most aerobic Gram-positive cocci, many anaerobic as well as microaerophilic and β-lactamase-producing Gram-negative and Gram-positive pathogens. Moreover, it is active against select protozoal organisms. It has been used for many years as prophylactic treatment during dental procedures to prevent endocarditis. In addition to its antiinfective properties, clindamycin has high oral absorption, significant tissue penetration, including penetration into bone, and stimulatory effects on the host immune system.

MEDICINAL USE

Lincomycin and clindamycin are clinically important antibiotics. According to its worldwide production, the semi-synthetic lincosamide derivative clindamycin belongs to twenty most important antibiotic compounds. Both antibiotics are therapeutically used especially in cases when synergistic effects of a mixed anaerobic and aerobic microflora are anticipated (prevention of intraabdominal infection after surgery, stomatological infections, anaerobic sepsis, skin and mucosa infections and especially bone and articular infections). As compared with lincomycin, clindamycin is more effective in the treatment of toxoplasmosis and pneumocystosis in AIDS patients.

Lincosamides are among the first choice bacteriostatic antibiotics used in veterinary dermatology. They are well absorbed if administered orally and penetrate well into infected skin. Their spectrum of action comprises bacteria commonly associated with skin infections, including staphylococci. Their main disadvantage is a rapid development of bacterial resistance and occasional gastrointestinal upset [1]. Clindamycin brings about changes in intestinal microflora and exhibits immunomodulatory effects [2]. According to some authors, care should be taken when using clindamycin for long-term and extensive treatment of other clinical situations that require antimicrobial intervention, due to its association with acute pseudomembranous colitis [3].

Some studies were devoted to examining the teratogenic potential for humans of antibiotics that can be used to treat routine and life-threatening infections during pregnancy and lactation. Treatment with clindamycin is considered compatible with breastfeeding [4].

Emerging clindamycin resistance may have serious implications in the treatment of severe infections caused by microbial pathogens [5]. One of the approaches to combat the ever increasing occurrence of microbial resistance (*see below*) is the use of combinations of different antibiotics, or of antibiotics with non-antibiotic antimicrobials.

Among the mechanisms of resistance is, e.g., the production by the microbes of enzymes degrading the antibiotic.

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Thus ampicillin, a β-lactam antibiotic, can be degraded by β-lactamase produced by the target organisms. Allewelt *et al.* [6] compared the treatment with ampicillin combined with the β-lactamase inhibitor sulbactam with the treatment with clindamycin in patients with aspiration pneumonia and primary lung abscess. Clindamycin was found to be a feasible option.

Also in antimalarial chemotherapy, the development of resistance has become a serious concern because resistance against most available drugs has developed in the majority of world-wide parasite populations. Combination products are again one of the ways to address this problem. A combination of clindamycin with the new antimalarial drug fosmidomycin was used for treating *Plasmodium falciparum* infections in pediatric patients [7, 8]. Asexual parasites were rapidly cleared in children treated with fosmidomycin-clindamycin and fosmidomycin alone but slowly in children treated with clindamycin alone. However, only treatment with fosmidomycin-clindamycin or clindamycin alone led to the radical elimination of asexual parasites. All regimens were well tolerated, and no serious adverse events occurred. However, relatively high rates of treatment-associated neutropenia (16%) and falls of hemoglobin concentrations are of concern. Efficacy appeared to be significantly reduced in children aged 1 to 2 years.

Like erythromycin and tetracycline, clindamycin used topically is bacteriostatic for *Propionibacterium acnes*, and has anti-inflammatory activities by inhibiting lipase production by *P. acnes*, and inhibiting leukocyte chemotaxis. In many patients with acne, caused by resistant *Propionibacterium acnes*, a continued treatment with antibiotics such as clindamycin can be ineffective [9]. Combination products often contain retinoids or benzoyl peroxide and the topical antibiotics; benzoyl peroxide is considered safe for use in pregnant and lactating females because it is degraded to benzoic acid [10]. Formulations such as the topical anti-acne drug combining clindamycin (1 %) and benzoyl peroxide (5%) in a stable, aqueous formulation (DuacR) [11] have been shown to (i) have antimicrobial, anti-inflammatory, and comedolytic effects and be more effective than either individual agent; (ii) exhibit a high efficacy and good to excellent tolerability of the combination; (iii) offer a simple, once-a-day application that encourages the compliance of acne patients; (iv) prevent the development of antibiotic resistance in acne patients; and (v) confer significant clinical improvement to patients who have already developed antibiotic resistance [12]. Benzoyl peroxide is a non-antibiotic antibacterial agent that is bactericidal against *P. acnes* and no resistance has been detected against it. Combined formulations such as clindamycin/benzoyl peroxide are highly effective, exhibit good overall tolerability and are useful in reducing the development of antibacterial resistance in *P. acnes* [13].

Ugwumadu [14] estimated the efficacy of oral clindamycin in eradicating abnormal vaginal flora in pregnancy and preventing relapse. The prevalence of abnormal flora post-treatment was 10% in the clindamycin group compared with 93% in the placebo group. At 20 weeks of gestation the prevalence of abnormal flora was 15% in the clindamycin group and 69% in the placebo group, and at 36 weeks of gestation the values were 17% in the clindamycin group and 43% at in the placebo group. Hence, oral clindamycin eradicated abnormal flora in 90% of treated pregnant women and maintained a normal flora in two thirds of women throughout pregnancy. In patients with bacterial vaginosis, Nyirjesy [15] determined clindamycin to promote similar levels of restoration of vaginal lactobacilli as metronidazole.

Necrotizing fasciitis is a life-threatening infection that spreads rapidly in the subcutaneous tissue, involving fascia superficialis. In addition to radical debridement, the therapy includes intravenous antibiotics, fluid and electrolyte management and analgesia. Clindamycin is among the discussed treatments, along with hyperbaric oxygen therapy and intravenous immunoglobulins [16].

Studies concerning adverse reactions of patients to clindamycin [17] showed high rates of hypersensitivity reactions to clindamycin in patients with acquired immunodeficiency syndrome (AIDS). These reactions have not been shown to be due to toxic metabolites of clindamycin and alternate mechanism(s) may be responsible for increased rates of adverse drug reactions to clindamycin among patients with AIDS. Recently, it has been found that prick and intradermal skin testing is not adequate in identifying patients with previous allergic reactions associated with clindamycin. Oral provocation tests can be used in patients with histories of clindamycin adverse reactions, albeit only on a risk-benefit basis [18].

Yunus [19] examined the relationship between the anticoccidial effects of clindamycin and the development of immunity in the *Eimeria pragensis* mouse model of large intestinal coccidiosis. A short-term (1 to 4 days or 4 to 8 days post infection) treatment schedules reduced clinical symptoms, oocyst production and schizogonic development, and allowed the development of a measurable protective immunity to the infection in the animals. In contrast, clindamycin treatment for the full 12-day period almost completely inhibited clinical symptoms but prevented the full development of protective immunity in the treated mice.

In dogs and cats, clindamycin was found to be almost completely absorbed after oral administration. Peak serum concentrations of this antibiotic are attained within 1-1.5 hours after dosing. It is widely distributed in many body fluids and tissues, and penetrates well into soft tissues, bones, joints, and the prostate gland. In dogs and cats clindamycin has been used in treatment of pyoderma, prostatitis, periodontal diseases, wounds and osteomyelitis caused by Gram-positive cocci or anaerobic bacteria [20].

Adjunctive use of clindamycin along with mechanical debridement has been recommended for the treatment of periodontitis associated with *Actinobacillus actinomycetemcomitans*, a Gram-negative, facultative anaerobic bacterium [21]. In the prophylaxis of orofacial infections of odontogenic origin encountered in immunocompromised patients, in individuals with endocarditis, vascular catheters or prostheses, or in other risk groups, clindamycin has been found to be an alternative drug to penicillin when the patients are allergic to penicillin [22, 23]. Clindamycin also plays an important role in treating infections caused by *Staphylococcus aureus*, group A beta-hemolytic streptococci and enteric organisms in children patients with a true allergy to penicil-

lins, who suffer from superficial (erysipelas, cellulitis, bullous impetigo, bite infections, and periorbital cellulitis) and deeper (orbital cellulitis, necrotizing fasciitis, and pyomyositis) infections of the skin and soft tissue [24].

Clindamycin is among the potential therapeutic options to treat community-associated infections by methicillin resistant *S. aureus* (MRSA). As compared with nosocomial infections, these infections are characterized by a lack of hospital-associated risk factors, improved susceptibility patterns, distinct genotypes, faster doubling times, and additional toxins [25].

CHEMISTRY AND BIOSYNTHESIS OF LINCOS--AMIDES

Lincomycin consists of an unusual amino acid, *viz. trans-N*-methyl-4-*n*-L-proline (propylhygric acid) linked by a peptide bond with the sugar 6-amino-6,8-dideoxy-1-thio-D-erythro-α-D-galactopyranoside (methylthio-lincosamide) [26, 27].

Like with other promising antibiotics, production of lincomycin by different *Streptomyces* species was marked by efforts to increase production yields and optimize the product by, e.g., producing the desirable lincomycin A without the concomitant production of lincomycin B [28-36].

Lincomycin B, *i.e.* 4´-depropyl-4-ethyllincomycin, appears often as a minority component in production cultures. Its antibiotic activity is a mere 25 % as compared with lincomycin A. Attempts at minimizing the amount of lincomycin B during the fermentation [37-39] included the addition of propylproline, a precursor of propylhygric acid in lincomycin A, to the cultivation medium. The mechanism by which the addition of propylproline or L-tyrosine and similar compounds influences the biosynthesis of lincomycin has not yet been clarified but N-demethyllincomycin synthetase, the enzyme catalyzing the linkage between the amino acid and sugar moiety of lincomycin, appears to utilize preferentially propylproline.

Another naturally occurring antibiotic of this group, celesticetin, with desalicetin, the alkaline hydrolysis product of celesticetin, were described [40] (Table **1**). Both compounds are less effective *in vitro* and *in vivo* than lincomycin against a number of microorganisms [41], although celesticetin exhibits a broad antibacterial spectrum, particularly against Gram-positive bacteria.

A number of lincomycin esters, either with organic (from acetate to stearate) or inorganic (phosphoric, carbonic) acids or of lincomycin alkyl derivatives (ethers), and salts of lincomycin with inorganic acids, *i.e.* hydrochlorides and derivatives of sulfamic acid, have been synthesized. Replacement of hydroxyl in the side chain (*i.e.* C-7 of octose) by chlorine yields clindamycin. Although hundreds of lincomycin derivatives were prepared including derivatives produced totally by chemical synthesis, clindamycin is the only drug that has been successfully used in the clinical practice. Chemical modification of lincomycin has yielded a number of derivatives with improved properties, whereas biotransformation has so far been less successful (Table **1**).

Lincomycin biosynthetic pathway proceeds *via* propylproline, the amino acid moiety and methylthio-lincosamide, the sugar moiety. Biosynthesis of propylproline begins with L-tyrosine, which is hydroxylated to yield L-3,4-dihydroxyphenyl alanine (L-DOPA). The subsequent steps include 2,3-extradiol cleavage of the L-DOPA aromatic ring, cyclization to produce the five-membered nitrogen containing ring, loss of two carbons, C-methylation at the aliphatic side chain and double reduction to yield propylproline. The C8 carbon skeleton of methylthiolincosamide apparently arises from condensation of a pentose unit (C5) and a C3 unit. The pentose unit could be derived either from glucose through the hexose monophosphate shunt as an intact unit or result from condensation of glyceraldehyde-3-phosphate with a C2 donor such as sedoheptulose-7-phosphate *via* a transketolase reaction. The final conversion of the C8-skeleton to methylthiolincosamide was postulated to involve isomerization and reduction of the C-8 carbon, transamination of the precursor 6-ketooctose, and final thiomethylation of the C-1 carbon. Propylproline and methylthiolincosamide then become condensed to *N*-demethyllincomycin, which is finally methylated to yield lincomycin (see Fig. **1**).

Whereas the amino acid part of the pathway has been partially clarified and some intermediates have been identified [42-44], no intermediates of the methylthiolincosamide part of the pathway have so far been conclusively identified.

ANTIMICROBIAL ACTIVITIES

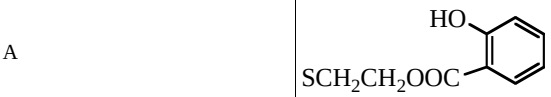
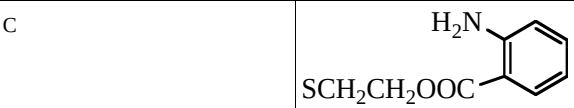
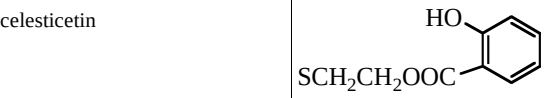
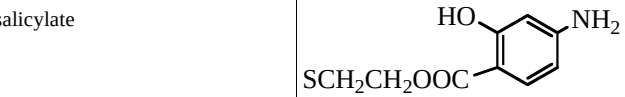
In usual doses both lincomycin and clindamycin exhibit bacteriostatic activity while at higher concentrations attainable *in vivo* they may exert bactericidal action. Clindamycin is generally more effective while lincomycin can be used in a substantially wider concentration range of clinical therapeutical doses.

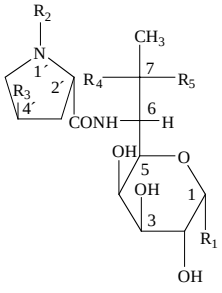
Table **2** gives the MIC values of lincomycin and clindamycin against some pathogenic bacteria. Clindamycin is active against most of the following bacteria: *Staphylococcus aureus*, *Streptococcus pyogenes*, *S. pneumoniae*, *S. viridans* and *S. bovis*, *Corynebacterium diphtheriae*, *Enterococcus durans*, *Bacillus anthracis*, *B. cereus* and the *Nocardia* spp. It can be used for the treatment of mouth infections caused by *Bacillus melaninogenicus* and *B. fragilis*.

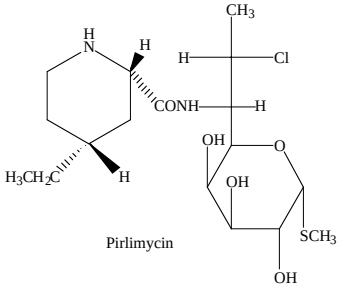
Bacterial skin infections, which are caused by aerobic streptococci and staphylococci (*Streptococcus pyogenies* and *Staphylococcus aureus*), with aerobic Gram-negative bacilli and anaerobes being involved in more complicated infections, are usually treated by systemic therapy with clindamycin [45].

Staphylococcus aureus and *Streptococcus pyogenes* cause a number of serious infections, such as necrotizing fasciitis and toxic shock syndrome, which are associated with the release of bacterial toxins. Animal studies showed that clindamycin is more effective in treating these severe infections than other drugs [46]. *Streptococcus pyogenes*, particularly the capsule and protein M, as well as streptococcal toxins cause severe septic and toxic syndromes. Clindamycin is used in cases of the septic shock [47]. Streptococcal toxic shock syndrome caused by, *e.g.*, *S. pyogenes*

Table 1. Structure of Lincomycin and Related Antibiotics

Name	R_1	R_2	R_3	R_4	R_5
Lincomycin A	SCH_3	CH_3	$CH_2CH_2CH_3$	OH	H
Lincomycin B	SCH_3	CH_3	CH_2CH_3	OH	H
Lincomycin C	SCH_2CH_3	CH_3	$CH_2CH_2CH_3$	OH	H
Lincomycin D	SCH_3	H	$CH_2CH_2CH_3$	OH	H
Lincomycin S	SCH_2CH_3	CH_2CH_3	$CH_2CH_2CH_3$	OH	H
Lincomycin K	SCH_2CH_3	H	$CH_2CH_2CH_3$	OH	H
Lincomycin sulfoxide		CH_3	$CH_2CH_2CH_3$	OH	H
1-Demethylthio-1-hydroxylincomycin	OH	CH_3	$CH_2CH_2CH_3$	OH	H
Celesticetin A		CH_3	H	OCH_3	H
Celesticetin B	$SCH_2CH_2OOCCH_2CH(CH_3)_2$	CH_3	H	OCH_3	H
Celesticetin C		CH_3	H	OCH_3	H
Celesticetin D	$SCH_2CH_2OOCCH_3$	CH_3	H	OCH_3	H
Desalicetin	SCH_2CH_2OH	CH_3	H	OCH_3	H
N-Demethylcelesticetin		H	H	OCH_3	H
Desalicetinsalicylate		CH_3	H	OCH_3	H
Clindamycin	SCH_3	CH_3	$CH_2CH_2CH_3$	H	Cl
Clindamycin sulfoxide		CH_3	$CH_2CH_2CH_3$	H	Cl
1´-demethylclindamycin	SCH_3	H	$CH_2CH_2CH_3$	H	Cl





Pirlimycin

can also be effectively treated by clindamycin and intravenous γ-globulin [48] while a combination of clindamycin and penicillin brings about enhanced bactericidal response against β-haemolytic streptococci [49].

Clindamycin has also been used to treat *Bacillus anthracis* infection [50], as well as pneumonia caused by *Bacillus cereus* [51].

Aerobic Gram-negative bacteria are in general resistant to clindamycin. *Campylobacter jejuni* is sensitive to clin-

Table 2. Effect of Lincomycin and Clindamycin on Some Pathogenic Bacteria

MIC values were obtained in a number of studies [52, 113-116] using different sets of strains and clinical isolates and different experimental approaches. This is reflected in various sizes of MIC ranges; the data serve only for general orientation.

Test organism	Minimum inhibitory concentration (μg/ml)	
	Lincomycin	Clindamycin
Bacillus anthracis	0.25–8.0	0.25–5.0
B. caccae		0.25– >128
B. fragilis		0.03– >128
B. thetaiotaomicron		0.5– >128
B. uniformis		0.5– >128
Bacteroides vulgatus		0.06– >128
C. perfringens		0.06–2
Campylobacter jejuni		0.125 - 2
Clostridium species		<0.03–4
Escherichia coli	1,000	64
Fusobacterium sp.		0.25– >128
Haemophilus influenzae	4–16	0.5–16.0
Klebsiella pneumoniae	8	125
Neisseria gonorhoeae	8–64	0.5–4.0
N. meningitis	>32	4
Peptostreptococcus sp.		0.06–_>128
Prevotella sp.		<0.03– >128
Proteus vulgaris	1,000	250
Pseudomonas aeruginosa	>1,000	1,000
Salmonella schottmuelleri	125	64
Staphylococcus aureus	0.2–3.2	0.04–1.6
Sta. epidermidis	0.4–1.8	0.1–0.2
Streptococcus agalactiae	0.1–0.2	0.02–0.1
S. pneumoniae	0.01–0.8	0.002–0.1
S. pyogenes	0.04–0.8	0.01–0.2
S. viridans	0.02–1.0	0.005–0.2
Veillonella sp.		<0.03– >128

Fusobacterium species includes F. mortiferum, F. necrophorum, F. nucleatum, F. varium and an unidentified Fusobacterium sp.; Prevotella species includes P. buccae, P. intermedia, P. melaninogenicus, P. oralis, and unidentified Prevotella spp.; Veillonella species includes V. parvulla and unidentified Veillonella spp. Peptostreptococcus species includes P. anaerobius, P. magnus, P. micros, and unidentified Peptostreptococcus spp.; Clostridium species includes C. clostridioforme, C. ramosum, C. butyricum, C. septicum and C. sporogenes.

damycin, while *C. coli* is much more resistant [52]. Clindamycin has good activity against the *Bacteroides fragilis* group of anaerobic bacteria although the number of clindamycin-resistant strains increases with time. It is quite active against other Gram-negative anaerobes such as *Prevotella disiens* and *P. melaninogenica* and the *Fusobacterium spp*. *Bacteroides gracilis* may be clindamycin-sensitive but some strains are resistant. Other Gram-negative bacteria comprising strains of *Butyrivibrio*, *Succinimonas* and *Anaerovibrio* can also be sensitive to clindamycin.

Among anaerobic bacteria, *Clostridium tetani* and *C. perfringens* are sensitive to clindamycin; but some *C. perfringens* strains and strains of *C. sporogenes*, *C. tertium*, *C. bifermentans*, *C. novyi*, *C. ramosum* and *C. sordelli*, may be clindamycin-resistant.

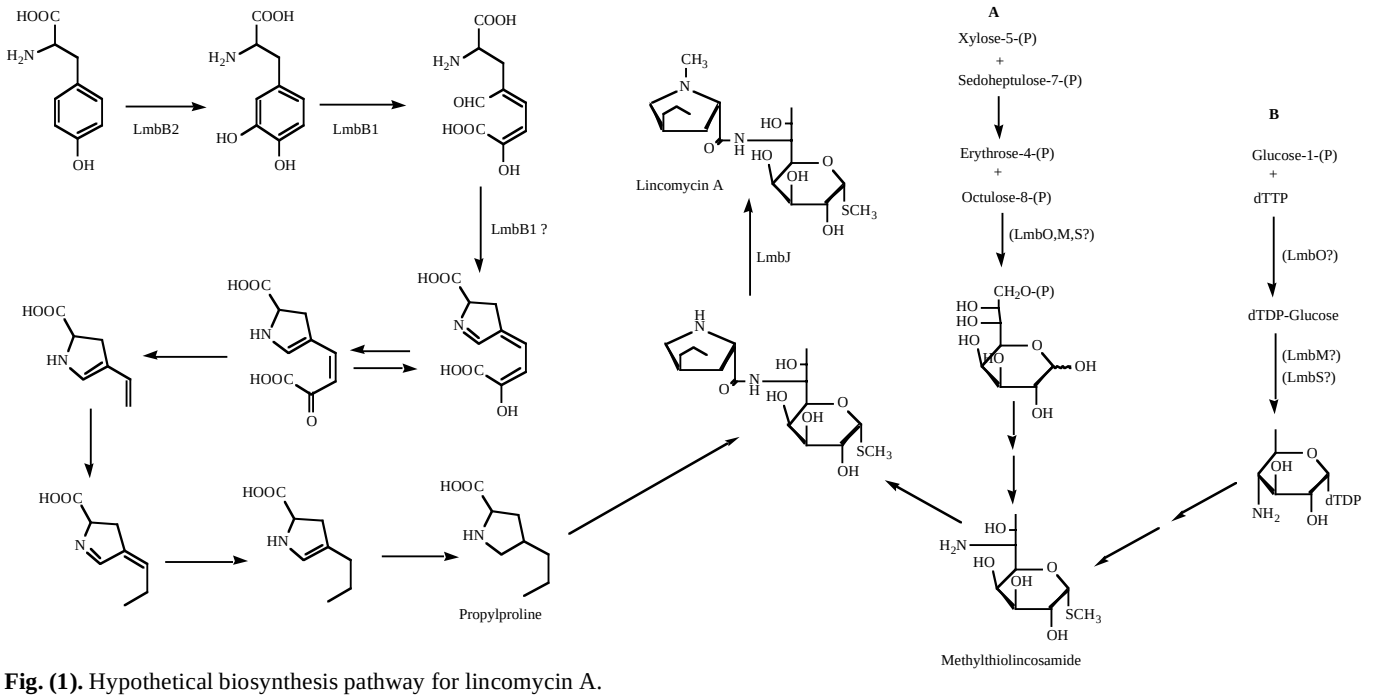


Fig. (1). Hypothetical biosynthesis pathway for lincomycin A.

Clostridium difficile is a major nosocomial pathogen, *C. difficile* infections occurring almost exclusively as a complication of antibiotic therapy, particularly that based on clindamycin and third-generation cephalosporins [53, 54]. *C. difficile* is responsible for numerous cases of diarrhea and colitis, the antibiotics most frequently associated with this infection including clindamycin, ampicillin, amoxicillin, and cephalosporins [55]. The association of clindamycin-like antibiotics with hospital-acquired *C. difficile* diarrhea was systematically reviewed by Thomas [56].

C. difficile may be clindamycin-sensitive or –resistant, the proportion of sensitive strains varying from 10% to 90% in different studies. During outbreaks of diarrhea associated with *C. difficile*, the strains are usually clindamycin-resistant and they contain a plasmid, probably located on the chromosome and coding for transferable MLSB resistance. This resistance can be transferred, *e.g*., from *C. difficile* to *Staphylococcus aureus*.

Clindamycin has been used as an effective drug in the treatment of Gram-positive anaerobic infections (*e.g.*, *Clostridium perfringens*) but an important decrease in bacterial susceptibility to clindamycin has recently been noted [57].

Other anaerobic Gram-positive organisms such as *Peptococcus, Peptostreptococcus, Eubacterium, Propionibacterium, Bifidobacterium* and *Lactobacillus* spp., *Actinomyces israelii* or *Bifidobacterium* and *Eubacterium* spp. are usually sensitive to clindamycin, although even here resistant strains were described as *Lactobacillus* spp. [58, 59].

Bacterial vaginosis is an alteration of the vaginal flora, where the normally predominant hydrogen peroxide-producing lactobacilli are replaced by high concentrations of a mixture of aerobic and anaerobic bacteria including *Gardnerella vaginalis*. It is treated with metronidazole or clindamycin but has been noted to occur in women treated, *e.g*., by orally administered clindamycin [60].

In cases of anaerobic sepsis, usually caused by *Bacteroides fragilis* or *Peptostreptococcus* sp., the application of clindamycin as the first choice antibiotic is fully justified. The management of bacteremia caused by anaerobic bacteria (*B. fragilis*, *Peptostreptococcus* sp., *Clostridium* sp., and *Fusobacterium* sp.) in children was reviewed [61].

Clindamycin was used as an antimalarial drug [62]. It was found effective in animals infected with chloroquine-resistant and -sensitive *Plasmodium falciparum*. It is also effective against *P. vivax*, but not against the exo-erythrocytic parasites.

In cultured mammalian cells clindamycin reduces the level of replication of *Toxoplasma gondii*, affecting protein synthesis of free parasites and also impairing the ability of the parasite to infect host cells. Infections caused by *T. gondii* can be treated with clindamycin [63].

Human babesiosis is an important emerging tick-borne disease caused by the cattle parasite *Babesia divergens* or *B. microti*. Human babesiosis can be treated by clindamycin administered intravenously [64], or by a combination of clindamycin and quinine [65].

GENETIC CONTROL OF LINCOMYCIN BIOSYNTHESIS

Like with other antibiotics produced by actinomycetes [66], genes coding for lincomycin biosynthesis are clustered together in a single genomic region and closely linked to the corresponding resistance determinants [67-70] (see Fig. **2**). According to Peschke [71] the cluster in *Streptomyces lincolnensis* contains 27 open reading frames with putative biosynthetic or regulatory functions (*lmb* genes) and three resistance (*lmr*) genes. The genes designated *lmr*A and *lmr*C flank the cluster and appear to code for proteins probably involved in lincomycin export [70, 71]. The *lmr*B gene codes for a protein very similar to several 23S RNA methyltrans-

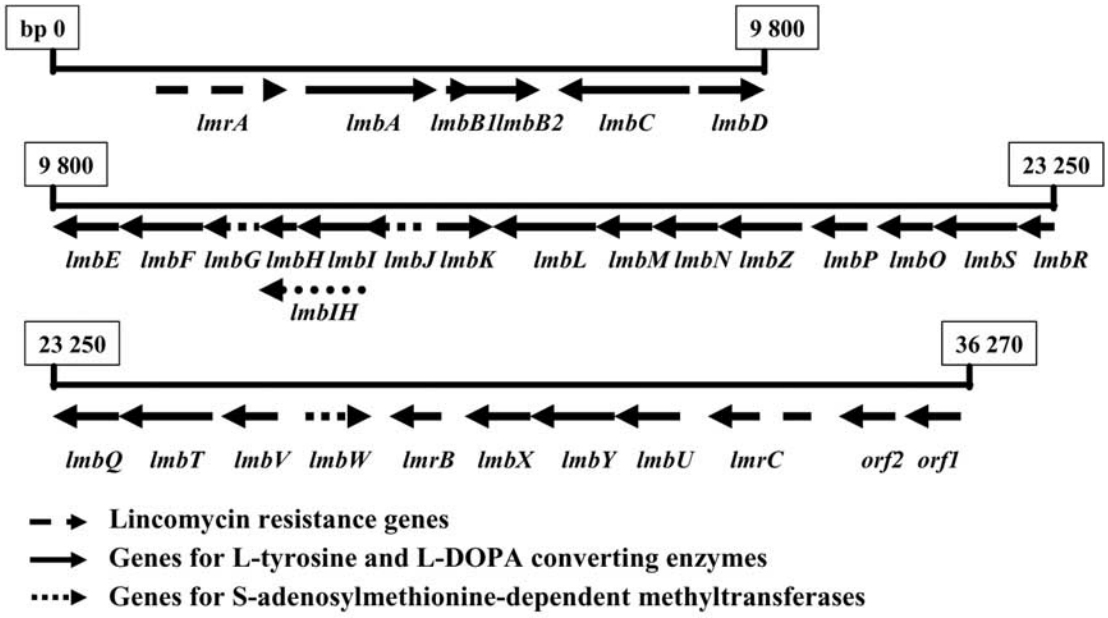


Fig. (2). The lincomycin gene cluster.

ferases [70]. The LmbB1 protein, participating in the biosynthesis of lincomycin, was heterologously expressed in *Escherichia coli*, purified in its active form, and characterized as a dimer of identical subunits [42]. Molecular mass and fragmentation pattern of the product revealed by capillary electrophoresis-mass spectrometry were in agreement with its proposed structure, 4-(3-carboxy-3-oxo-propenyl)-2,3-dihydro-1H-pyrrole-2-carboxylic acid. The LmbB1 was therefore found to be a dioxygenase catalysing the 2,3-extradiol cleavage of the L-3,4-dihydroxyphenyl alanine aromatic ring. The final LmbB1 reaction product, a unique compound found in biosynthesis of lincomycin and expected in anthramycins, arises through subsequent cyclization of the primary cleavage product, 2,3-secodopa.

It is assumed that genes coding for enzymes involved in propylproline synthesis are located close to *lmr*A gene whereas those controlling methylthio-lincosamide synthesis are located further from it [69]. The genes *lmbB1* and *lmbB2* code for enzymes converting L-tyrosine and L-dihydroxyphenylalanine [72], gene *lmbJ* apparently codes for a specific N-demethyllincomycin methyltransferase [73]. Genes *lmbL* through *lmbQ* form a subcluster which probably codes for a set of enzymes involved in sugar metabolism [71]. Putative proteins LmbO, LmbM and LmbS show similarity to enzymes involved in the central steps of many NDP-6-deoxyhexose pathways, including sugar-activating pyrophosphorylases (LmbO), NDP-hexose dehydratases (LmbM), and (NDP-) ketosugar (or cyclitol) aminotransferases/dehydratases (LmbS). The corresponding genes are found mostly in clusters in both Gram-positive and Gram-negative bacteria and their protein products are involved in the formation of other actinomycete secondary metabolites [74-77] or of extracelullar polysaccharides [78].

MECHANISM OF ACTION ON CELLS

Lincosamides belong to antibiotics that block microbial protein synthesis. A molecular mechanism by which clindamycin inhibits ribosomal protein biosynthesis in prokaryotic microorganisms is associated with the fact that clindamycin´s three dimensional structure closely resembles L-Pro-Met and the D-ribosyl ring of adenosine [79], which occur near one another at the 3´-ends of L-Pro-Met-tRNA and deacylated-tRNA for a brief interval following the formation of a peptide bond between L-Pro-tRNA and L-Met-tRNA. Hence clindamycin and other lincosamides may act as structural analogues of the 3´-ends of L-Pro-Met-tRNA and deacylated-tRNA during an initial phase of pretranslocation in the peptide elongation cycle. Although the chemical structure of macrolides (*e.g.* erythromycin), lincosamides (*e.g.* lincomycin, clindamycin and celesticetin) and streptogramins is very different, their mechanism of action is similar. Erythromycin binding with the 23S rRNA blocks polypeptide translation, resulting in a release of peptidyl-tRNA intermediates prematurely by blocking the approach to the elongating peptide´s exit tunnel [80]. Although the macrolides in general do not directly block the peptide bond-forming step at the peptidyltransferase center of the 50S subunits, it is known that they compete with lincosamide antibiotics that are direct peptidyltransferase inhibitors. Clindamycin and erythromycin binding shows a partial physical overlap. Lincomycin and clindamycin share a common mechanism of action on sensitive microorganisms and also exhibit a similar antibacterial spectrum. Nevertheless, slight differences in their antimicrobial activity exist as clindamycin also affects some protozoa, *e.g. Toxoplasma gondii*, *Plasmodium falciparum* and *Pneumocystis carinii*.

Clindamycin inhibits bacterial protein synthesis and acts specifically on the 50S subunit of the bacterial ribosome by affecting the process of peptide chain initiation. It may also stimulate dissociation of peptidyl-tRNA from ribosomes [81]. Lincomycin was also used as inhibitor of protein synthesis in plants and algae [82, 83].

IDENTIFYING LINCOSAMIDES AND THEIR METABOLITES

Part of the assessment of the therapeutic action of lincosamides, their toxicity, side effects and the action of lincosamide degradation products or metabolites is an efficient separation and identification of the antibiotics or their fragments in tissues and body fluids. The last two decades saw the development of instrumental methods based on liquid chromatography, mostly on reversed-phase C18 columns, as a separation technique and UV-spectroscopy or soft ionization techniques of mass spectrometry as an identification method.

Lincomycin and related antibiotics were analyzed in bovine milk extract by a MS/MS/CID (collision-induced dissociation) technique, which gave an on-column lincomycin detection limit of 0.8 pg [84]. A reverse-phase ion-pair liquid chromatography with a base-deactivated column and pulsed electrochemical detection by means of a gold electrode was used for the analysis of lincomycin and spectinomycin in two commercial samples [85].

A sensitive method using liquid chromatography and electrospray ionization tandem mass spectrometry (LC-ESI/MS/MS) was used for determining lincomycin in animal tissues [86]. An HPLC method based on reversed phase separation (C18 column) and photodiode-array detection has been developed for the simultaneous determination of tretinoin and clindamycin phosphate, and their degradation products in topical formulations [87]**.** Another HPLC method for the quantitative determination of clindamycin in animal serum made use of UV detection. The assay was validated for a concentration range from 80 to 6000 ng/ml serum [88]**.** High-performance liquid chromatography on a cyano column with UV detection at 204 nm was also employed for the assay of clindamycin in human plasma [89].The method was specific and sensitive with a lower limit of quantitation of 0.2 μg/ml. In the stability test, clindamycin was found to be stable in human plasma during the storage and assay procedure.

Rapid identification of clindamycin and its related minor impurities in bulk drug was achieved by high-performance liquid-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS) [90]. The ESI-MS results served the authors to propose plausible schemes for their fragmentations, which were confirmed further by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry using collision-induced dissociation method at high mass resolution. The positive ESI-MS/MS of clindamycin and its derivative compounds showed some diagnostic fragments, such as the neutral losses of H_2O, HCl, methanethiol and 2-methylthio-ethenol, and the residue of 3-propyl-N-methylpyrrolidine and 3-ethyl-N-methylpyrrolidine, which are specific and useful for the identification of the lincosamide antibiotics and related impurities.

Sin [91] described a simple, selective and sensitive method for simultaneous determination of lincomycin and virginiamycin M-1 in swine muscle and organs using liquid chromatography with electrospray ionization tandem mass spectrometry. Analytes in purified acetonitrile extracts were separated on a reversed-phase C-18 column.

A method for the quantification of clindamycin in animal plasma using LC-MS/MS/ESI has also been published by Cherlet [92]. The limit of quantification of the method was 50 μg/ml, the detection limit 1.3 ng/ml. The method was used for pharmacokinetic studies of clindamycin formulations in dogs. Liquid chromatography-electrospray ionization tandem mass spectrometry was also used to determine residues of lincomycin and tylosin in honey as part of a field study of treatment of honey bees with these antiobiotics [93]. Honey samples were diluted and injected directly into the LC-MS/MS system without additional cleanup by solid-phase extraction or liquid-liquid partitioning. The method detection limits were determined to be 5 and 2 μg/kg for lincomycin and tylosin, respectively.

The crystal structure of lincomycin hydrochloride monohydrate was determined by Rajeswaran and Srikrishnan [94] in order to obtain the conformational and structural features of the drug and permit a comparison of its structural features with other aminoglycoside antibiotics (Fig. **3**). The absolute configuration was established using the anomalous dispersion of the sulfur and chlorine atoms in the structure. The molecule consists of an amino acid linked by an amide group to a monosaccharide of galactose stereochemistry. A network of hydrogen-bonds stabilizes the crystal structure. Since according to X-ray crystallographic data the methyl thiolincosaminide portion of lincomycin and the desosamine sugar unit in erythromycin occupy virtually identical sites on the 23S rRNA, the synthesis of 3-N,N-dimethylamino-3-deoxy lincomycin as a hybrid structure was performed in eight steps from lincomycin, involving a *trans*-diequatorial opening of an intermediate epoxide as the key step [95].

An interesting study described a rapid clean-up procedure based on solid-phase extraction and HPLC determination of lincomycin in premixes with UV detection at 208 nm. Premix extracts were applied to column treated with methanol and water [96]. Lincomycin was eluted with methanol and the effluent was analyzed on analytical column (phenyl) using mobile phase consisting of phosphoric acid in water and acetonitrile. The limit of determination, based on a signal-to-noise ratio of 10:1, was 5.2 mg/kg. LC-MS/MS confirmation of lincomycin was performed by monitoring two pairs of multiple reaction monitoring ions from the parent ions.

Among other methods, capillary electrophoresis was used for a simultaneous determination of lincomycin and four other aminoglycoside antibiotics (kanamycin, netilmicin, tobramycin, and amikacin). Under optimum separation conditions, the aminoglycoside antibiotics were baseline separated within 20 min and the detection limit was below 6.7 μM for lincomycin [97].

MICROBIAL RESISTANCE TO LINCOSAMIDES

Bacteria can acquire resistance to lincosamides and other antibiotics - macrolides, streptogramin antibiotics - by modification of the target site of the drugs, by active efflux of the

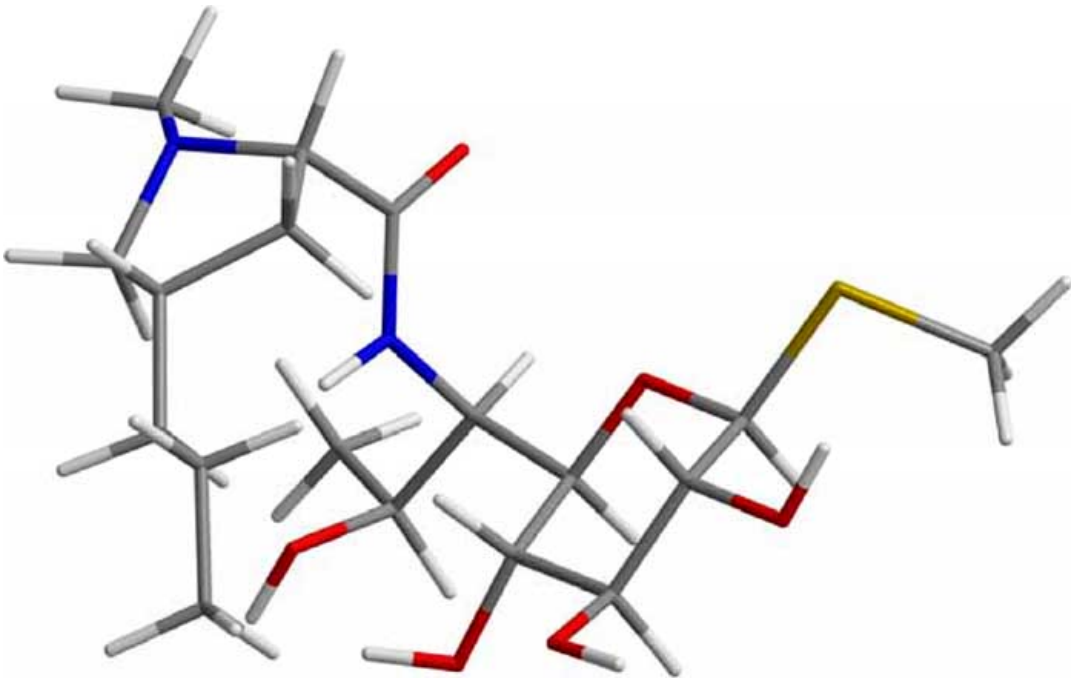


Fig. (3). 3D model of lincomycin molecule.

drugs, and by inactivation of the drugs. In general, basic mechanisms of antibiotic resistance include microbial cell impermeability, target site modification, enzymatic modification or destruction of the antibiotic and its increased efflux. Because of the increasing emergence of resistance, major efforts are devoted to combat the resistance by combining lincosamides with other agents (*see above*). The genetic background of drug resistance is well described and methods for genotypic susceptibility testing have been developed [98].

The rise of macrolide- and lincosamide-resistant strains of pathogenic Gram-positive cocci over the past decades has changed treatment guidelines and induced the researchers to detect new resistance mechanisms that may profoundly affect the clinical outcome. The rise and dissemination of common macrolide and lincosamide resistance mechanisms that have been identified in *Staphylococcus* and *Streptococcus* species of clinical interest have been discussed in detail by [99].

The main type of resistance to lincomycin and clindamycin is the so-called MLSB resistance which renders sensitive microorganism resistant to macrolides, lincosamides and streptogramin B. It is monomethylation or dimethylation of the N_6 exocyclic amino group of A2058 by specific ribosome methylation modification enzymes. This type of resistance is associated with genes encoding methyltransferases modifying the common target site of macrolides and lincosamides, *i.e.* 23S ribosomal RNA (*e.g.* genes *ermA* and *ermC*). A specific gene was also described, whose protein product modifies and thus inactives lincosamide antibiotics (*linA*). Lincosamide resistant *Streptococcus pneumoniae* carries mutations in the 23S rDNA with substitutions at A2058, A2059, or C2611 and in L4 or L22 ribosomal protein genes. 50S Ribosomal mutations are the least frequent mechanism of *S. pneumoniae* resistance [100]. Other, less common, phenotypes arise from other 23S rRNA modifications (ML and K phenotypes) or from amino acid substitution (MSB phenotype) or insertion (MKSB pheotype) into the 50S subunit ribosomal protein L4 [101].

Kehrenberg [102] and Long [103] reported on a new mechanism for chloramphenicol, florfenicol and clindamycin resistance, viz. methylation of 23S ribosomal RNA at A2503 by the *cfr* gene product from *Staphylococcus sciuri*, *S. aureus* and *E. coli*. The results show that *Cfr* is an RNA methyltransferase that targets nucleotide A2503 and causes resistance to chloramphenicol, florfenicol and clindamycin by inhibiting ribose methylation at nucleotide C2498. *S. aureus* and *E. coli* strains expressing the *cfr* gene exhibit elevated MICs to a number of chemically unrelated drugs. The phenotype was named PhLOPSA for resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A, important antimicrobial agents that are currently used in human and/or veterinary medicine. Binding of the PhLOPSA drugs, which bind to overlapping sites at the peptidyl transferase center next to nucleotide A2503, is perturbed upon *Cfr*-mediated methylation. No other rRNA methyltransferase is currently known to confer resistance to five chemically distinct classes of antimicrobials.

As described by Rich [104], resistance to clindamycin in methicillin-resistant *Staphylococcus aureus* may be due to modification of the ribosomal target site. Resistance due to this mechanism is encoded by the *errs* genes, which may be either constitutively or inducibly expressed. Inducible resistance is not usually revealed by routine laboratory susceptibility testing but can be detected by a relatively simple double disc agar diffusion test. Since staphylococci can develop resistance to clindamycin even during treatment when inducible resistance mechanisms are present, routine screening for inducible resistance is recommended.

Lincomycin resistance in clinical isolates of staphylococci and streptococci has been recognized for several decades. This resistance is plasmid mediated and is encoded on transposons. The resistance results from the induction of an enzyme that is normally repressed. The methylated RNA binds lincomycin-type drugs less well than does the non-methylated RNA. The induction of resistance varies by species, and in most Gram-positive species, erythromycin is a more effective inducer of resistance than clindamycin. The plasmids that mediate lincomycin resistance in streptococci and staphylococci are highly similar structurally indicating that they could have been readily transferred among strains of these species. Loeza-Lara [105] described pBMSa1, a cryptic plasmid from a dairy cow isolate of *S. aureus*, that encodes a lincomycin resistance determinant and replicates by the rolling-circle mechanism

As part of a study of macrolide and lincosamide resistance genes, Loch [106] tested environmental streptococcus isolates from bovine milk for resistance to erythromycin and pirlimycin by broth microdilution assays. Pirlimycin is a lincosamide antibiotic with activity primarily against gram-positive organisms, including *Staphylococcus* and *Streptococcus* species. It is considered more active than clindamycin against *S. aureus.* Pirlimycin is not active against Gram-negative bacteria, such as *E. coli.* Resistance to erythromycin and pirlimycin in *S. dysgalactiae* and *S. uberis* isolates was encoded by *ermB*. All streptococcus isolates tested negative for *ermA, ermC, mefA/E* and *msrA/C*. The authors suggest a theoretical potential for horizontal transfer of macrolide resistance genes on dairy farms.

Other microorganisms such as lactobacilli isolated at chicken farms [107] (*L. crispatus, L. salivarius* subsp. *salivarius*, *L. amylovorus*, *L. gallinarum* and *L. reuteri*) showed a very high prevalence of acquired resistance to macrolides and lincosamides The vast majority of these resistant strains (96%) displayed constitutive resistance. More than one-half of the macrolide and/or lincosamide resistant strains were found to carry an *ermB, ermC, mefA, lnuA* gene or a combination of these genes.

Distribution of macrolide, lincosamide, streptogramin, ketolide and oxazolidinone (MLSKO) resistance genes in Gram-negative bacteria was studied by Roberts [108]. Some of the acquired genes are thought to be unique to Gram-negative bacteria, some are shared with Gram-positive bacteria and some are primarily of Gram-positive origin. In addition, mutations, which modify the 23S rRNA, ribosomal proteins L4 and/or L22, and/or changes in expression of innate efflux systems which occur by missense, deletion and/or insertion events have been described in five Gram-negative groups, while an innate transferase conferring resistance to streptogramin A has been identified in a sixth genus. The interaction between members of the MLSKO antibiotic family and Gram-negative bacteria changes the resistance to these antibiotics by mutations of existing genes, by acquisition and perhaps mutations of acquired resistant genes in these organisms.

Increased efflux of lincosamides was detected in some microorganisms resistant to them. Active efflux of the antibiotic from the periplasmic space mainly occurs in Gram-negative bacteria [109].

Methicillin-resistant strains of *Staphylococcoccus aureus* possess *ermC* and *ermA* as the most frequent determinants of MLSB resistance (up to 90 % cases); gene *msrA*, encoding a protein responsible for the active excretion of macrolides and streptogramins but not of lincosamides by the resistant cells, is less common. Gene *linA*, whose protein product modifies and thus inactivates lincosamide antibiotics only, is an additional resistance gene that is less frequent [110].

Inactivation (resistance) of lincosamides by the products of the *linA* (encoding 3-lincomycin 4-clindamycin O-nucleotidyltransferase) genes of *Staphylococcus aureus* is one of the resistance mechanism in this bacterium [111].

Stepanovic [112] studied the resistance profiles of the *Staphylococcus sciuri* group members *S. sciuri, S. lentus*, and *S. vitulinus* to macrolides, lincosamides, streptogramins (MLS antibiotics), and linezolid. The study involved the PCR detection of the resistance genes *ermA, ermB, ermC, mefA, lnuA*, and *lnuB*. Resistance mediated by active efflux was detected in one strain, the presence of genes *ermB* or *ermC* was detected in two strains and the *lnuA* gene was detected in two strains. The great majority of the tested *S. sciuri* strains seemed to exhibit LSA phenotype: they did not carry *lnu* genes nor displayed constitutive MLSB resistance, but still showed intermediate resistance or resistance to lincomycin. The results indicate that *S. sciuri* may be naturally resistant to lincomycin.

CONCLUSION

Lincosamide antibiotics, in particular clindamycin, are therapeutically used especially in cases of infections involving mixed anaerobic and aerobic microflora, in particular Gram-positive bacteria, and also protozoal infections. They are frequently employed as topical or systemic dugs in dermatology and stomatology, for the treatment of vaginoses, anaerobic sepsis and other complaints, and in veterinary medicine. They exhibit immunomodulatory effects. A promising trend in lincosamide therapy that was successful, e.g., in the treatment of malaria, is their combination with other, either antibioti or non-antibiotic, antimicrobials such as cephalosporin, fosfidomycin or benzoyl peroxide. The development of new instrumental methods based mostly on liquid chromatography as a separation technique and UV-spectroscopy or soft ionization techniques of mass spectrometry as an identification method spurred studies of the possible effects of lincomycin or clindamycin impurities and/or metabolites on the therapy and potential hypersensitivity reactions, especially in immunocompromised patients.

The main type of resistance to lincomycin and clindamycin, the so-called MLSB resistance, renders sensitive microorganisms resistant to macrolides, lincosamides and streptogramin B due to monomethylation or dimethylation of the amino group of A2058 or A2503 of 23S ribosomal RNA by specific ribosome methylation enzymes. Resistance phenotype PhLOPSA, i.e. resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A, is associated with *cfr* genes, constitutively or inducibly expressed resistance to clindamycin in some bacteria is encoded by the *errs* genes. Bacteria of different genera that display constitutive resistance often carry an *ermA,ermB, ermC, mefA, lnuA* or *linA* gene or a combination of these genes.

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