RESEARCH AND REVIEWS: JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Farewell, Chloramphenicol? Is this True?: A Review.

Madhavan HN*, and Bagyalakshmi R.

Larsen and Toubro Microbiology Research Centre, Kamal Nayan Bajaj Research Centre, Vision Research Foundation, No.41, College Road, Chennai – 600006, Tamil Nadu, India.

Review Article

ABSTRACT

Chloramphenicol has remained as a potent broad spectrum antibiotic over decades and due to its side effects its usage has been limited. In an era of increasing resistance to many antibacterial agents, chloramphenicol might have a role in the treatment of intra abdominal infections and respiratory tract infections caused by multi drug-resistant pathogens. This review article focuses on the spectrum of activity of chloramphenicol, mechanism of action, side effects, its parentral and oral use, its resistance mechanisms, toxicity elucidated by enteric pathogens against it and also on the possibility of its therapeutic use in treatment of multi - drug resistant bacterial infections.

INTRODUCTION

During the last decade the emergence of highly resistant pathogens has aroused renewed interest in older drugs that have fallen into disuse because of several factors including toxic side effects. One important antibiotic agent is chloramphenicol, which was released for use in the United States in 1949, but due to reports linking its use with the development of aplastic anemia it has long been put aside and is not used as standard therapy in the developed world, though it is still considered first-line treatment for enteric fever and other infections in many developing countries.[1]

Chloramphenicol, a potent inhibitor of protein synthesis, is extremely active against a variety of organisms including bacteria, spirochetes, rickettsiae, chlamydiae and mycoplasmas. It has bacteriostatic activity against most pathogens but is bactericidal for *Haemophilus influenzae, Streptococcus pneumoniae*, and *Neisseria meningitides.*[2]

Respiratory pathogens such as *H .influenzae* and *S. pneumoniae* have also retained high susceptibility rates, with 99.2% and 99.4%. [3,4] The high susceptibility rates noted for chloramphenicol might be due to the very limited use of this drug for many years in the developed world. However, the use of chloramphenicol has reduced over a period of time due to the adverse effects like bone marrow depression or in some cases severe aplastic anaemia. [5]

Despite the constantly increasing need for new antimicrobial agents, their discovery and development appear to have greatly reduced in recent years. Presented with the significant problem of advancing antimicrobial resistance, the global scientific community has attempted to find alternative solutions; one of the most promising ones is the evaluation and use of old antibiotic compounds which have been placed under orphan drugs. Due to the low-level use of many of the older antibiotic compounds, these have remained active against a large number of currently prevalent bacterial isolates. Thus, clinicians are beginning to re-evaluate their use in various patient populations and infections, despite the fact that they were previously thought to be less effective and/or more toxic

than newer agents. A number of older antibiotic compounds, such as polymyxins, fosfomycin, fusidic acid, sulphonamides, aminoglycosides and chloramphenicol, are re-emerging as valuable alternatives for the treatment of difficult-to-treat infections. The availability of novel genetic and molecular modification methods provides hope that the toxicity and efficacy drawbacks presented by some of these agents can be surpassed in the future. In an era of increasing resistance to many antibacterial agents, chloramphenicol might have an important role in the treatment of intra abdominal infections and respiratory tract infections caused by multi drug-resistant pathogens. This review article focuses on the spectrum of activity of chloramphenicol, mechanism of action , side effects , its parental and oral use, its resistance mechanisms, toxicity elucidated by enteric pathogens against it and also on the possibility of its therapeutic use in treatment of multi - drug resistant bacterial infections.

Discovery and Synthesis

Chloramphenicol was originally derived from the bacterium *Streptomyces venezuelae,* isolated by David Gottlieb, and introduced into clinical practice in 1949, under the trade name chloromycetin and was widely used in the 1950s. It was the first antibiotic to be manufactured synthetically on a large scale and is a potent broad spectrum antibiotic. Contributing substantially to its biological activity is a dichloroacetyl substituent, the biochemical derivation of which remains obscure.[6] The pathway for chloramphenicol biosynthesis was initially deduced from the patterns of isotope incorporation into chloramphenicol produced when cultures were fed with labeled substrates, and from the structures of metabolites accumulated by blocked mutants.[7,8] The results indicated a plausible series of biosynthetic reactions that generated chloramphenicol by dichloroacetylation of an aromatic intermediate.

Spectrum of action

Chloramphenicol acts on the protein synthesizing system of bacteria (the cell's ribosomes) yet does not affect mammalian, reptilian, or avian ribosomes. Due to its pH, it has the ability to penetrate into infected tissues and tissues with biological barriers. Chloramphenicol can easily pass deeply through cell membranes to inactivate bacteria living within, and into organs where other antibiotics cannot. Highly susceptible bacteria are killed outright while others are merely rendered unable to divide and the host's immune system then destroys them upon discovery.

Because it functions by inhibiting bacterial protein synthesis, chloramphenicol has a very broad spectrum of activity: it is active against Gram-positive bacteria (including the dreaded strains of MRSA), Gram-negative bacteria and anaerobes. It is not active against *Pseudomonas aeruginosa, Chlamydiae,* or *Enterobacter* species. It has some activity against *Burkholderia pseudomallei*, but is no longer routinely used to treat infections caused by this organism (it has been superseded by ceftazidime and meropenem).

Because of its excellent blood brain barrier penetration (far superior to any of the cephalosporins), chloramphenicol remains the first choice treatment for staphylococcal brain abscesses. It is also useful in the treatment of brain abscesses due to mixed organisms or when the causative organism is not known. Chloramphenicol is active against the three main bacterial causes of meningitis: *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae*. Chloramphenicol has been used in the initial empirical treatment of children with fever and a petechial rash, when the differential diagnosis includes both *Neisseria meningitidis* septicaemia as well as Rocky Mountain spotted fever, pending the results of diagnostic investigations. Chloramphenicol is also effective against *Enterococcus faecium*, which is being considered for treatment of vancomycin-resistant enterococcus. [9]

Mechanism of action

Chloramphenicol binds to the 50S ribosomal subunit and blocks fundamental ribosomal functions, such as peptidyltransferase (PTase) activity, binding and movement of ribosomal substrates through the PTase center and translation termination.

Experiments from more than several years ago have demonstrated that both monovalent and Mg^{2+} ions at optimal concentrations ^{2} are essential components for the PTase activity and the interaction of chloramphenicol with ribosomes. [10] Vogel et al,[11] have explained about the rationale for the interdependence of monovalent/ Mg^{2+} ions and chloramphenicol interaction with ribosomes. As proposed by Bayfield et al, [12] this ion is dissociated in inactive ribosomes and this dissociation might also account for the inability of inactive ribosomes to bind chloramphenicol.

Side effects

The major side effects found in patients treated with chloramphenicol include irreversible bone marrow depression, aplastic anemia, and leukemia. Epidemiologic studies indicate that 1:30,000 to 1:45,000 patients receiving

chloramphenicol treatment go on to develop leukemia and based on epidemiologic studies, chloramphenicol has been strongly correlated with leukemogenesis.[13] Chloramphenicol-mediated hematotoxicity manifests as either a reversible, predictable, dose-dependent, early-onset, mild anemia characterized by reticulocytopenia with occasional leucopenia and thrombocytopenia observed during therapy or a post treatment pancytopenia that is unpredictable, irreversible, dose independent, and occasionally fatal.[14] Aplastic anemia appears to have ancillary factors such as underlying mitochondrial dysfunction, genetic polymorphisms that accentuate chloramphenicol binding to mitochondrial rRNA , or a genetic predisposition that enhances the ability of the bone marrow to nitroreduce chloramphenicol into its myelotoxic derivative. Chloramphenicol associated aplastic anaemia cannot be predicted by the monitoring of blood cell counts.[15] The percent mortality of chloramphenicol associated aplastic anaemia is around 50% and in neonates it 40 %.[16] Prognosis is poor, if aplastic aneamia develops after a long time of treatment. Only orally administered chloramphenicol leads to aplastic anaemia. This has made chloramphenicol to be prescribed parenterally by many physicians. It is not known whether this lowers the incidence of aplastic anemia or not but the risk is obviously lowered.[17]

Another side effect of chloramphenicol is the grey baby syndrome which is a rare condition almost exclusively seen in neonates and very young infants. Toxic blood levels of chloramphenicol secondary to neonatal hepatic enzyme immaturity leads to circulatory collapse and the signs of cardiac shock. The infant is cyanosed, is acidotic, has cold peripheries and has the signs of all of marked hyponia, poor feeding, vomiting, loose stools and a distended abdomen. Because of this, it is recommended that chloramphenicol not been to be given to neonates or to young infants, but if given should be administered in low doses (25 mg/kg/day in four divided doses). [18] The adverse side effects of chloramphenicol are tabulated in Table 1. These adverse effects have led to dramatic reductions in the use of chloramphenicol in developed country settings. Use of chloramphenicol has been replaced by other antibiotics with a better side effect profile, although it remains a useful agent. In resource poor settings, its use has continued because of its availability and low cost.

Table 1: Dose related adverse effects of chloramphenicol

Abnormalities	Dose related adverse side effect
Haematologic	Bone marrow suppression, haemolytic aneamia
Neurological	Optic neuritis, peripheral neuritis, headache, encephalopathy, mental confusion, depression
Other	Hypersensitivity reactions, nausea, vomitting, diarrhoea, pseudomembranous colitis, glossitis, stomatitis, ototoxicity

Phramacokinetics

Three preparations of chloramphenicol are most commonly used in clinical practice: a crystalline powder for oral administration, a palmitate ester for oral administration as a suspension, and a succinate ester for parenteral administration. Both esters are inactive, requiring hydrolysis to chloramphenicol for anti-bacterial activity. The palmitate ester is hydrolysed in the small intestine to active chloramphenicol prior to absorption. The bioavailability of oral crystalline chloramphenicol and chloramphenicol palmitate is approximately 80%. The time for peak plasma concentrations is dependent on particle size and correlates with in vitro dissolution and deaggregation rates. The bioavailability of chloramphenicol after intravenous administration of the succinate ester averages approximately 70%, but the range is quite variable. Incomplete bioavailability is the result of renal excretion of unchanged chloramphenicol succinate prior to it being hydrolysed to active chloramphenicol. Plasma protein binding of chloramphenicol is approximately 60% in healthy adults. The drug is extensively distributed to many tissues and body fluids, including cerebrospinal fluid and breast milk, and it crosses the placenta. Reported mean values for the apparent volume of distribution range from 0.6 to 1.0 L/kg. Most of a chloramphenicol dose is metabolised by the liver to inactive products, the chief metabolite being a glucuronide conjugate; only 5 to 15% of chloramphenicol is excreted unchanged in the urine. The elimination half-life is approximately 4 hours. Inaccurate determinations of the pharmacokinetic parameters may result by incorrectly assuming rapid and complete hydrolysis of chloramphenicol succinate.[19]

Parenteral vs oral administration of chloramphenicol

Chloramphenicol can be given orally or parenterally but the bioavailability of oral formulations is better than that achieved after intramuscular or intravenous injection of the succinate ester pro-drug. [20] Nevertheless, parenteral dosing is indicated in patients with nausea, vomiting and/or altered consciousness. Although an inadequate therapeutic response has been reported after intramuscular administration,[21] more studies in enteric fever have suggested that peak plasma chloramphenicol concentrations are not influenced significantly by the route of parenteral administration.[22]After intravenous or intramuscular chloramphenicol succinate injection, approximately one-third of the dose is excreted unchanged in the urine while the remainder is hydrolysed to free drug and either excreted in the urine or conjugated in the liver.[23]

In order to characterize the factors affecting chloramphenicol pharmacokinetics after its parenteral administration as treatment for enteric fever, Acharya et al, [24] studied the disposition of chloramphenicol after intravenous or intramuscular injection of the succinate ester, together with simultaneous estimates GFR (iothalamate clearance), hepatocellular function (antipyrine clearance) and liver blood flow (ICG clearance).The findings of the study revealed that intravenous chloramphenicol produced peak plasma concentrations which are on average twice those after intramuscular injection of the same dose.

The recommended route of administration of chloramphenicol is usually oral for the treatment of typhoid fever. The intravenous route is used with severely ill patients, in whom absorption may be poor. In adults, chloramphenicol can be given as the base orally, and absorption is rapid and complete. The intravenous preparation is chloramphenicol succinate, an inactive precursor, which is hydrolyzed to chloramphenicol in the body. In a study on healthy volunteers given equivalent doses of chloramphenicol, Glazko et al.,[20] reported that the area under the plasma concentration-time curve of chloramphenicol given intravenously as chloramphenicol succinate was only 60 to 70% of that obtained with oral chloramphenicol. Teow –Yee et al.,[25] conducted a study to measure the serum chloramphenicol and chloramphenicol succinate concentrations in patients with typhoid fever who were being treated with either oral chloramphenicol or intravenous chloramphenicol succinate and to assess the clinical outcome of the treatment. In this study, the authors assessed serum chloramphenicol and chloramphenicol succinate concentrations in patients given equivalent doses of chloramphenicol base either intravenously or orally for typhoid fever by high-performance liquid chromatography. The mean serum chloramphenicol concentrations were significantly lower in the patients treated with intravenous chloramphenicol succinate than in the patients treated with oral chloramphenicol capsules. The study emphasizes that where ever facilities are available, it may be prudent to measure serum chloramphenicol concentrations, especially in patients who are treated with intravenous chloramphenicol succinate, to ensure that failure to respond is not due to low serum drug concentrations. The significant findings, revealing the outcomes of orally administered chloramphenicol is given in Table 2.

Table 2: Significant findings revealing the outcomes of orally administered chloramphenicol

Studies	Significant findings	Reference
Oral chloramphenicol treatment *H. influenzae* meningitis	(1) chloramphenicol can be used by the oral route to complete treatment of *H. influenzae* type b meningitis; (2) a dose of 75 mg/kg/day is effective and less likely than higher doses to cause neutropenia; and (3) the measurement of serum chloramphenicol concentrations is important, regardless of route of administration.	Tuomanen et al [26]
Oral chloramphenicol in the therapy of *H. influenzae* meningitis	Chloramphenicol levels in the cerebrospinal fluid (CSF) following oral administration of chloramphenicol palmitate were as high, or higher, than those following i. v. therapy with chloramphenicol succinate.	Yogev et al [27]
Pharmacokinetic Comparison of Intravenous and Oral Chloramphenicol	The mean serum half-life of the drug (6.5 hours) was significantly longer after oral administration than after intravenous chloramphenicol (4.0 hours) (P .001).	Yogev et al [28]
Comparison of ofloxacin vs oral chloramphenicol in treatment of typhoid fever	Three days ofloxacin was more effective than 14 days chloramphenicol for the in-patient treatment of typhoid fever, irrespective of antibiotic susceptibility, and was of similar cost	Phongmany et al [29]
Comparison of ceftriaxone, with 14-day chloramphenicol therapy	This study suggests that a short treatment of three days of typhoid fever with ceftriaxone (3 or 4 g once daily) is adequate and not hazardous as far as relapses are concerned.	Lasserrea et al [30]
Randomized Controlled Trial Comparing Ciprofloxacin and Chloramphenicol Treatments against Enteric Fever	Ciprofloxacin was more effective in the elimination of *Salmonella enterica serovars typhi* and *paratyphi* A from bone marrow than chloramphenicol, there was still an impressive persistence of Salmonella in the bone marrow culture (67%). In the ciprofloxacin-treated patients the suppressed cytokine production capacity showed a trend to normalize earlier than in patients treated with chloramphenicol.	Gasem et al [31]

Ophthalmic chloramphenicol

Topical chloramphenicol remains popular partly because of its wide spectrum of action, being highly effective against most Gram positive and Gram negative pathogens as well as most anaerobes (including all obligate anaerobes). It has a high reported efficacy of 91% to 93% in ocular infections [32] and is active against up to 94% ocular pathogens.[33] Chloramphenicol also has a high differential solubility giving high intraocular penetration

(10 000 times more fat soluble than penicillin), making it superior to most other antibiotics for prophylaxis in ocular surgery, with penetration of ointment noted to be superior to eyedrop preparations (single application of 1% ointment as effective as 13 applications of eyedrop preparation every 15 minutes).[34]

Adverse reactions

Topical chloramphenicol is generally very well tolerated with uncommon local adverse effects of hypersensitivity and transient burning/stinging. [35] A study conducted by Wallerstein et al [36] revealed that marrow toxicity may not manifest until the second or third course of treatment. The usual adult systemic dose of chloramphenicol is 3 to 5 g per day in divided 4 hourly doses while one drop (0 05 ml) of guttae chloramphenicol 0-5% contains 0-25 mg of chloramphenicol meaning that a typical course of topical treatment would provide a total of less than 10 mg delivered to .the eye, with only a fraction of this amount being absorbed systemically. There are several studies revealing the ophthalmic use of chloramphenicol in the management of ocular infections .The key findings of these studies are given in Table 3:

The debate over the safety of topical chloramphenicol has caused a widespread response in the ophthalmic literature. The article by McGhee and Anastas,[44] has reviewed the knowledge and concluded that there is a theoretical but not conclusively proved risk of aplastic anaemia in the use of topical ophthalmic chloramphenicol.

The authors quoted an idiopathic aplastic anaemia incidence of 1 in 524 000, or 2 per million in the USA. The use of chloramphenicol in Europe is 40 times the amount of that in the USA and reflects the different prescribing habits between the two communities. If topical ocular chloramphenicol was to have a relation to aplastic anaemia the incidence in Europe will be expected to be higher than in the USA. This issue was addressed by Gardner, 1991 who quoted an incidence of 1.5 per million cases of aplastic anaemia in France, a similar figure to that of the USA. These data suggest that there is no difference in the incidence of aplastic anaemia when topical ocular chloramphenicol is prescribed compared with when it is not. Practitioners should continue to use this effective drug until there is firm evidence that it is not safe.[45]

Table 3: Ophthalmic use of chloramphenicol

Ocular infection	Key findings	References
Bacterial keratitis	18% of bacterial strains resistant to chloramphenicol	Schaefer et al [37]
Conjunctivitis	OTC Chloramphenicol in the treatment of adenovial conjunctivitis	Elton [38]
Ofloxacin vs chlormaphenicol in the management of external ocular infection	Microbiological improvement rates were similar for the two drugs: 85% (33/39) improved with ofloxacin, and 88% (38/43) improved with chloramphenicol. Adverse reaction encountered was 1% with ofloxacin as against 4% with chlormaphenicol	Bron et al [39]
Treatment of *Chlamydia trachomatis* infection with rifampicin and chloramphenicol	4- to 6-week course of treatment with 1 % chloramphenicol eye ointment 3 times a day appeared to reduce the severity of clinical signs and symptoms slightly but did not cure the infection nor did it eliminate the *C. trachomatis*.	Darougar et al [40]
Chloramphenicol treatment for acute infective conjunctivitis in children	The numbers of children treated with chloramphenicol who showed bacterial eradication (40%) differed significantly to those treated with placebo No resistant isolates were detected in the study.	Rose et al [41]
Effectiveness of 2.5% povidone-iodine eye drops (PIED) compared with ophthalmic chloramphenicol (OC) in neonatal conjunctivitis	During the first 48 hours after birth, PIED and OC had similar efficacy against bacterial conjunctivitis. From day 3 to day 15, PIED was 6% less effective than OC. PIED seemed to increase the risk of acquiring chlamydial conjunctivitis	Ramirez-Ortiz et al [42]
Treatment of *Pseudomonas aeruginosa* contact lens-associated keratitis with topical chloramphenicol	0.5% Chloramphenicol had been used in one-third of cases of P. aeruginosa contact lens associated keratitis prior to the use of a broad-spectrum antimicrobial, which was associated with more complications and a longer interval to resolution, but with no adverse effect on final visual acuity	Bourkiza et al [43]

Clinical usage of chloramphenicol

Chloramphenicol is of undoubted value for the treatment of typhoid fever and bacterial meningitis. With the exception of neonatal forms of meningitis, a combination of penicillin G and chloramphenicol is the best 'antibiotic cover' before the causative organism is identified. Chloramphenicol is very effective for the treatment of meningitis due to *Haemophilus influenzae* type b. [44,45,46,47] Parenterally administered ampicillin, previously regarded by many as equally effective to chloramphenicol for this infection, is no longer dependable. Chloramphenicol may be

preferable for the treatment of other severe *H. influenzae* type b infections such as epiglottitis, osteomyelitis, cellulitis and pneumonia.

The best substitute drug for the treatment of meningococcal, pneumococcal or *Listeria monocytogenes* meningitis in penicillin-allergic patients is chloramphenicol.[48] This may also apply to meningitis caused by Group B streptococci. Chloramphenicol together with penicillin G is often the preferred chemotherapy for the treatment of cerebral abscess. Chloramphenicol is especially useful for temporal lobe abscesses of otitic origin which usually yield a mixed bacterial flora including anaerobes such as *Bacteroides fragilis* .[49] Metronidazole or clindamycin may prove to be acceptable alternative drugs for this clinical situation. By comparison to ampicillin and cotrimoxazole, chloramphenicol appears superior for the treatment of typhoid fever.[50] For the treatment of paratyphoid fever and septicaemia caused by other Salmonella spp., chloramphenicol is also probably the most effective drug; alternative drugs for these diseases are the same as for typhoid. One of these alternatives must be used when chloramphenicol-resistant *Salmonella* species strains are involved. Strains of Salmonella typhi resistant to chloramphenicol caused a typhoid epidemic in Mexico during the early 1970's, but whenthe epidemic subsided endemic strains reverted to chloramphenicol sensitivity. [51] Chloramphenicol resistant *S. typhi* and other salmonella, strains appear to be prevalent in South-East Asia. [52]

Chloramphenicol in treatment of bacterial meningitis

Bacterial meningitis is still a major public health problem in developing countries. Three species of bacteria *Haemophilus influenzae* type b (Hib), *Neisseria meningitidis* (Mnc), and *Streptococcus pneumoniae* (Pnc) cause more than three-quarters of all cases of acute bacterial meningitis in such countries. Schauf et al.,[53] have conducted a study to determine the bactericidal effects of chloramphenicol and three, β-lactams (ampicillin, cefamandole, and penicillin G) on 27 strains of *Haemophilus influenzae* type b isolated from the blood or cerebrospinal fluid of infected infants. Of the ampicillin-susceptible strains, 75% were killed by < 2.0 ug of each antibiotic per ml; however, the concentration of the β -lactam agents required for bactericidal activity was higher than that required for inhibitory activity. Chloramphenicol was the only agent which had no marked discrepancy between inhibitory and bactericidal concentrations regardless of β -lactamase production.

Pecoul et al [54] have conducted a clinical trial to compare the efficacy at day 4 of a double intramuscular injection of long-acting chloramphenicol 100 mg/kg with that of ampicillin administered intravenously for 8 days at 200 mg/kg (4 times a day). The results did not demonstrate any statistically significant difference between long-acting chloramphenicol and ampicillin with respect to case fatality rates and failure rates at day 4. The authors have concluded that long-acting chloramphenicol is cheap and easy to administer, advantages that are of particular importance where medical resources are scarce, and it is now admitted that adverse effects are unusual. The use of a single injection of an oily suspension of chloramphenicol to patients of all ages with meningococcal meningitis has been recommended.[55] In most field conditions, the bacteriological diagnosis of meningitis is not feasible. Starting antibiotic treatment as early as possible for all suspected cases of bacterial meningitis is the only means of preventing death and secondary morbidity. For such a strategy, an oily suspension of chloramphenicol appears to be the best antibiotic to use as a first-line treatment; a second injection of oily chloramphenicol has to be given no more than 48 hours later. After 3 or 4 days, depending on clinical or biological criteria, the treatment can be modified or continued with oral chloramphenicol.

Rahal et al [56] have studied the bacteriostatic and bactericidal effects of chloramphenicol, ampicillin, tetracycline, and sulfisoxazole and compared it against several potential meningeal pathogens. Chloramphenicol is bactericidal at clinically achievable concentrations against *Haemophilus influenzae, Streptococcus pneumoniae*, and *Neisseria meningitidis*. It is bacteriostatic against gram-negative bacilli of the family Enterobacteriaceae and against *Staphylococcus aureus*. Chloramphenicol has proven highly efficacious in the treatment of bacterial meningitis caused by those organisms against which it is bactericidal at low concentrations. The authors concluded that because leukocytic phagocytosis in the subarachnoid space is inefficient, the bactericidal activity in cerebrospinal fluid is important for optimal therapy of bacterial meningitis. Chloramphenicol does not provide such activity in meningitis caused by enteric gram-negative bacilli. The significant findings of various studies conducted with regard to the use of chloramphenicol in the treatment of bacterial meningitis is provided in Table 4

Table 4: Chloramphenicol in the treatment of bacterial meningitis

Bacterial meningitis	Key findings	References
Chlormaphenicol trial in Northern Savanna, Africa	In a controlled trial chloramphenicol proved as effective and much cheaper than pencillin for the treatment of group A meningococcal meningitis in Zaria, Nigeria. Chloramphenicol is a suitable alternative to sulphonamides for the treatment of mningococcal meningitis	Whittle et al [57]
Combined action of chloramphenicol and ampicillin on chloramphenicol-resistant *Haemophilus influenzae*	Initial treatment of meningitis caused by ampicillin-susceptible, chloramphenicol-resistant strains, inhibition of the action of ampicillin by chloramphenicol may represent a clinical risk.	Mackenzie et al [58]
Meningitis due to beta lactamase producing type b *Haemophilus influenzae* resistant to chloramphenicol	Cases of meningitis due to strains of type b Haemophilus influenzae resistant to both ampicillin and chloramphenicol have been reported in Great Britain. Prompt recognition of such resistance is important, the rapid demonstration of chloramphenicol acetyltransferase production should be routinely applied to strains of *H influenzae* isolated from patients with meningitis.	Garvey et al [59]
In-vitro and in- vivo efficacy of ceftriaxone, moxalactam, and chloramphenicol against *Haemophilus influenzae* type b	Ceftriaxone is much more effective than moxalactam or chloramphenicol in the treatment of H. influenzae type b sepsis and meningitis	Connor et al [60]
Chloramphenicol and penicillin resistance in Pneumococci	6.9%isolates of pneumococci showed relative resistance to penicillin, 2.0% were resistant to chloramphenicol Screening all isolates from invasive pneumococcal infections for penicillin and chloramphenicol susceptibility recommended	Istre et al [61]
Antagonism of ceftazidime by chloramphenicol in vitro and in vivo during treatment of Gram negative meningitis	Definite and clinically important antagonism, both in vitro and in vivo, between chloramphenicol and the cephalosporin ceftazidime observed during treatment of a young infant with salmonella meningitis. The combination treatment failed to eradicate the infection but subsequent treatment with ceftazidime alone was successful.	French et al [62]
Haemophilus influenzae type b resistant to ampicillin and chloramphenicol.	Two cases of meningitis due to *Haemophilus influenzae* type b resistant to ampicillin and chloramphenicol has been documented In one child the meningitis was preceded by pneumonia and pleural effusion. Both children responded to treatment with cefotaxime.	Solaranzo et al [63]
Therapeutic efficacy of chloramphenicol, co-trimoxazole (trimethoprim/sulphamethoxazole), cefmenoxime and ceftriaxone in experimental bacteraemia and meningitis caused by ampicillin-resistant Haemophilus influenzae type b	Chloramphenicol was effective in reducing mortality, but failed to eradicate the organism or to prevent relapse, while co-trimoxazole was least effective in that all but one survivors suffered relapse with positive blood and CSF cultures. Ampicillin gave unexpected results in that the organism was eradicated in all survivors and bacteriological relapse was prevented in most animals	Kim et al [64]
Chloramphenicol or ceftriaxone, or both, as treatment for meningitis in developing countries	Using a third generation cephalosporin as first line treatment is effective in dealing with the problem of poor outcomes from meningitis due to *Haemophilus influenzae* that is resistant to chloramphenicol, and a strategy of changing to chloramphenicol if in vitro susceptibility is shown will reduce the use of expensive third generation cephalosporins without comprising on clinical outcomes.	Duke et al [65]

Table 5: Chloramphenicol in the treatment of typhoid fever

Chloramphenicol in the treatment of typhoid fever	Key findings	References
Chloramphenicol in typhoid -fever a preliminary report of clinical trial in 6 cases	Six culture-positive typhoid cases successfully treated with chloramphenicol Chloramphenicol strikingly alters the course of typhoid fever by exerting a specific therapeutic action.	Patel et al [70]
Comparison of chloramphenicol and ampicillin in the treatment of typhoid fever	The comparative effectiveness of treatment with chloramphenicol and ampicillin revealed that chloramphenicol was rapid in action as compared to ampicillin.The temperature persisted for 3.6 days in chloramphenicol patients and for 6.1 days in ampicillin patients.	Scioloi et al [71]
Chloramphenicol as a drug of choice in the treatment of typhoid	In an eight year study 0.47% of *S. typhi* strains were resistant to chloramphenicol Chloramphenicol remains a satisfactory first-line choice of drug for typhoid fever in Britain.	Rowe et al [72]
Comparative evaluation of amoxicillin and chloramphenicol in the treatment of typhoid fever in Africa	Amoxycillin was associated with a shorter time to defervescence, fewer complications and lower mortality when compared to chloramphenicol, but the differences were not statistically significant Amoxycillin appears to be at least as effective as,and possibly associated with fewer complications than, chloramphenicol in the treatment of typhoid.	Ellis et al [73]
Treatment of typhoid fever with ceftriaxone for 5 days or chloramphenicol for 14 days: a Randomized Clinical Trial	A short, 5-day course of ceftriaxone is a useful alternative to conventional 14-day chloramphenicol therapy in the treatment of typhoid fever.	Islam et al [74]
Comparison of aztreonam and chloramphenicol in the treatment of typhoid fever	Aztreonam was less effective than chloramphenicol with regard to clinical effectiveness and time of defervescence but was more effective in the elimination of the infecting Salmonella organisms from the bloodstream.	Gotuzzo et al [75]
The concurrent prevalence of chloramphenicol-sensitive and multidrug-resistant *Salmonella typhi* in Vellore	A multidrug resistant (MDR) variety of *Salmonella typhi* emerged as the cause of epidemic typhoid fever from April 1990 to the first quarter of 1993. In the same period, to chloramphenicol sensitive (CS) *S. typhi* also. were isolated. The relative prevalencesshowed that the frequency of CS variety was unaffected by the epidemic of MDR variety. This is an unusual epidemiological pattern, which indicates that there may have been factors which favoured the epidemic of the MDR variety but not the CS one.	Jesudason et al [76]
Treatment of typhoid fever with azithromycin versus chloramphenicol	Azithromycin given once daily for 7 days was an effective therapy for typhoid fever in a region endemic with chloramphenicol-resistant *S. typhi* infection and was equivalent in effectiveness to chloramphenicol given to patients with chloramphenicol-susceptible infections	Butler et al [77]
Randomized controlled trial comparing ciprofloxacin and chloramphenicol treatments against enteric fever	this study and the cumulative literature show that rates of clinical cure of patients with entericfever after 7 days of ciprofloxacin and 14 days of chloramphenicol are similar. However, the studies are not sufficient to demonstrate that ciprofloxacin is equivalent or superior to chloramphenicol.	Gasem et al [78]
Clinical and microbiological investigations oftyphoid fever	37% were MDR strains indicating the emergence of highly resistant *Salmonella enterica var typhi*	Dimitrov et al [79]
Ceftriaxone versus chloramphenicol for treatment of acute typhoid fever	Marked reduction of the prevalence of MDR *Salmonella typhi* isolates and marked increase in the susceptibility of these isolates to chloramphenicol, returning it to be one of the drugs that could be used in the treatment of acute typhoid fever	Hammad et al [80]
Preserving efficacy of chloramphenicol against typhoid fever	A decrease in the incidence of multidrug resistant *Salmonella typhi* along with an increase in non-multidrug resistant isolates of same organism. This is most likelydue to reduced use of traditional antimicrobial agents (ampicillin, chloramphenicol, co-trimoxazole) and increasing reliance on ciprofloxacin as the first line of treatment of patients with typhoid fever.	Harish et al [81]
Antimicrobial susceptibility of *Salmonella typhi* serovars In Southern India	Among the 322 clinical isolates recovered from blood culture 57.8% were *S. typhi*, 41.6%, were *S.paratyphi* A and 2 were *S. paratyphi* B . 90% of the isolates were susceptible to ampicillin, 95% to cotrimoxazole and 100% to chloramphenicol while 13.66% of isolates exhibited resistance to ciprofloxacin and 91.9% to nalidixc acid.	Choudhary et al [82]

Typhoid fever (caused by *S. typhi*) remains a significant problem and is estimated to have caused 21.6 million illnesses and 216,500 deaths globally, affecting all ages. Chloramphenicol no longer has a place as first line treatment of *Salmonella typhi* (S typhi) as much of it in the developing world is now resistant to chloramphenicol. Multiple resistance (to chloramphenicol, ampicillin and cotrimoxazole) has been reported in up to 90% of cases of *Salmonella typhi* in Vietnam and almost 70% in Pakistan. [66] It has been suggested that almost all countries now have a significant proportion of multiple resistance of this bacteria. The current drugs of choice are fluoroquinolones and third-generation cephalosporins, but decreased susceptibility to these antimicrobials has also been reported.[67]

After sporadic outbreaks of chloramphenicol resistant typhoid between 1970 and 1985, many strains of *S typhi* developed plasmid mediated multidrug resistance to the three primary antimicrobials used (ampicillin, chloramphenicol, and co-trimoxazole). This was countered by the advent of oral quinolones, but chromosomally acquired quinolone resistance in *S typhi* and *S paratyphi* has been described in various parts of Asia, possibly related to the widespread and indiscriminate use of quinolones.

Appropriate antibiotic treatment (the right drug, dose, and duration) is critical to curing typhoid with minimal complications. The emergence of multidrug resistant typhoid in the 1990s led to widespread use of fluoroquinolones as the treatment of choice for suspected typhoid, especially in South Asia and South East Asia where the disease was endemic.[68]

In the past few years, however, the emergence of resistance to quinolones has placed tremendous pressure on public health systems in developing countries as treatment options are limited.[69] The review of the use of chloramphenicol in treatment of typhoid fever is provided in Table 5

Chloramphenicol resistance

Resistance to chloramphenicol is mainly due to the production of inactivating enzymes, the chloramphenicol acetyl transferases (CAT). Three types of CAT (types I–III) have been described in gram-negative bacteria, but resistance due to the production of CAT of type I has been encountered most frequently in Enterobacteriaceae and is generally plasmid-encoded. However, there are also reports on other mechanisms of chloramphenicol resistance, such as efflux systems inactivation by phosphotransferases mutations of the target site and permeability barriers.

Chloramphenicol acetyltransferases (CATs) are able to inactivate chloramphenicol as well as thiamphenicol and azidamfenicol. Due to the replacement of the hydroxyl group at C-3 by a fluor residue, the acceptor site for acetyl groups was structurally altered in Ff. This modification rendered Ff resistant to inactivation by CAT enzymes, and consequently, chloramphenicol-resistant strains, in which resistance is exclusively based on the activity of CAT, are susceptible to Ff . There are two defined types of CATs which distinctly differ in their structure: the classical CATs, referred as type A CATs and the novel CATs, also known as xenobiotic CATs and referred as type B CATs.

Apart from enzymatic inactivation, which confers chlomphenicol resistance transposon based chloramphenicol resistance was first detected in 1979 in *P. aeruginosa* and later on shown to be based on the presence of the transposon *Tn1696*.[83]

Emergence of chloramphenicol resistance

Until the mid-1970s chloramphenicol was the undisputed drug for treatment of typhoid fever, and in developed countries the use of this antibiotic resulted in a reduction in mortality from 10% to <2% . Although before 1970 a few sporadic isolations of chloramphenicol-resistant *Salmonella typhi* had been reported from chloramphenicol-resistant strain was that which occurred in Mexico in 1972. At about the same time, there was a second substantial outbreak in Kerala, India [84] and in both outbreaks mortality was high. In the succeeding 5 years outbreaks occurred in several other countries, notably, Vietnam, Indonesia, Korea, Chile, and Bangladesh.[85] A notable feature of all chloramphenicol-resistant strains from outbreaks in Mexico, the Indian subcontinent, and Southeast Asia was that although the strains belonged to different Vi phage types, resistance to chloramphenicol was encoded by a plasmid of the H 1 incompatibility group, often in combination with resistance to streptomycin, sulfonamides, and tetracyclines. [86]

In India, the incidence of MDR *Salmonella typhi* isolates has been reported to be as high as 60% in Pune ,[87] in 1999, but then declining to 22% in Nagpur.[88] The resurgence of resistant isolates in Ludhiana, India, in 2002 has, however, been a cause for concern [89,90]. A US-based study of imported strains [91] noted an increase in the number of MDR and Nalidixic acid resistant *Salmonella typhi* globally, although all isolates remained sensitive to ciprofloxacin and ceftriaxone. In Bangladesh[92] there has been a reported decrease in MDR isolates with no

corresponding increase in sensitive strains. For ciprofloxacin there has been an increase in minimum inhibitory concentration in strains imported into the UK,[93] in Bangladesh,[94] as well as in India. [95]

DISCUSSION

Chloramphenicol has been efficient in treating not only enteric fever and meningitis but also pulmonary infections. In assessing the role of chloramphenicol in treatment of acute respiratory tract infection, Ioannidis et al [96] have evaluated the efficacy of chloramphenicol in treatment of acute respiratory tract infection of mixed bacterial origin and reported that a clinical improvement was encountered in 96.25% patients. In an other study by Kemmerich et al [97] the authors evaluated the use of enoxin, ofloxacin, ampicillin and chlormaphenicol for treatment of experimental *Haemophilus influenzae* pneumonia and reported a survival rate as 48% with chlormaphenicol as against 78% with ampicillin. Howard et al [98] have reported that 1.9% strains of *Haemophilus influenzae* type b as resistant to both ampicillin and chlormaphenicol which in turn suggests that cefotaxime to be used as the first line empirical treatment for *Haemophilus influenzae* type b pulmonary infection. In yet another study by Deivanayagam et al [99] the authors evaluated the effectiveness of ampicillin and combination of penicillin and chlormaphenicol in treatment of pneumonia and reported that there is no significant difference observed in the primary outcome, cure rate or secondary outcome among the two groups. With regard to community acquired pneumonia, Asghar et al [100] have reported that there is treatment failure with chloramphenicol in children aged between 2 -59 months .Aspa et al [101] have reported that 18% of *Streptococcus pneumoniae* causing pneumonia in adults in Europe exhibited resistance to chlormaphenicol . Campos et al [102] 2003 have reported that 14.3% of *Haemophilus influenzae* type f were resistant to chloramphenicol. Duke at al [103] have emphasized the fact that WHO recommends the use of chloramphenicol in neonates who fail to respond to first line antibiotics

The role of chloramphenicol in the treatment of Vancomycin resistant enterococcus blood stream infection is well documented. and mortality rate was lowered inpatients treated with chloramphenicol.. Hartmann et al.,[104] have reported the first paediatric case of ventriculitis due to VRE (in a 2-month-old infant) to be successfully treated with combined intravenous (i.v.) and intraventricular chloramphenicol after failure of i.v. linezolid and intraventricular gentamicin. Sood et al.,[105] have reported a case of prosthetic valve endocarditis and persistent bacteremia due to vancomycin-resistant *Enterococcus faecium*. The combination of parenteral chloramphenicol plus minocycline therapy was administered for 8 weeks and resulted in cure after treatment with quinupristin-dalfopristin had failed. Chloramphenicol proved to be effective in initiating a clinical response in multi drug resistant bacterial infections

Loss of R-plasmid would lead emergence of chloramphenicol-sensitive strains showing very low MICs. High degree of chloramphenicol susceptibility to S. enterica serovar typhi isolates has also been reported from India Over period of years several Indian studies have documented a 90-95% re-emergence of chloramphenicol susceptibility. This has also been reported even from neighboring countries. Since its introduction in1948, chloramphenicol has remained the drug of choice for typhoid fever and remains the standard against which the newer antimicrobials are compared. Treatment with chloramphenicol in typhoid fever reduces the mortality from 20 to 1% and the duration of fever from 14-28 to 3 to 5 days. Chloramphenicol has re-emerged as valuable drug of choice for treatment of enteric fever in India.

Recently, the New Delhi metallo-beta-lactamase (NDM-1), a novel type of metallo-beta-lactamase (MBL) conferring resistance to almost all beta-lactam antibiotics, including carbapenems that are used in treatment of antibiotic-resistant bacterial infections, has emerged and, therefore, has alarmed the world. This has become a significant threat to human health due to its extensive drug resistance, leaving few or no therapeutic options and, therefore, becoming a major public health problem throughout the world, particularly in India. Chloramphenicol has the potential to induce a good clinical response even in bacterial infections caused by microbes harbouring blaNDM-1 in the future.

CONCLUSION

The reduced use of chloramphenicol appears to increase the sensitivity of chloramphenicol. The reversal may be due to loss of plasmids encoding resistance to chloramphenicol and other first line drugs like ampicillin, co-trimoxazole or may be due to emergence of susceptible strains. Chloramphenicol can be administered orally and its clinical efficacy is well established.Where drug sensitivity tests are not available, chloramphenicol can be given in uncomplicated typhoid fever and the response monitored carefully. In fact investigators have suggested using chloramphenicol, along with the third-generation cephalosporins, in typhoid fever due to ciprofloxacin-resistant *S. enterica serovar typhi* infection. In view of Re-emergence of sensitivity and reduced resistance to chloramphenicol in *S. typhi* isolates in many studies, chloramphenicol may be reconsidered for the treatment as the antibiotic of choice for the treatment typhoid fever.

In an era of increasing resistance to many antibiotics, chloramphenicol might have a role in the treatment of intraabdominal infections and respiratory tract infections caused by multidrug-resistant pathogens. Thus, it represents a great comeback in clinical practice.

REFERENCES

1. Nitzan O, Suponitzky U, Kennes Y, Kennes, Chazan B, Raz R et al Is chloramphenicol making a comeback? Isr Med Assoc J. 2010; 12: 371-74
2. Mir SA, Abbas Z. Chloramphenicol : A come back. JK Science. 2010; 12: 153
3. Hoban DJ, Doern GV, Fluit AC, Roussel-Delvallez M, Jones RN. Worldwide prevalence of antimicrobial resistance in *Streptococcus pneumoniae, Haemophilus influenzae*, and *Moraxella catarrhalis* in the SENTRY. Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis. 2001; 32 (Suppl 2): S81-93.
4. American Academy of Pediatrics, Subcommittee on Management of acute otitismedia. Diagnosis and management of acute otitis media. Pediatrics 2004; 113: 1451-65.
5. Li CH, Cheng YW, Liao PL, Yang Y, Kang J. Chloramphenicol causes mitochondrial stress, decreases ATP biosynthesis, induces matrix metalloproteinase-13 expression, and solid-tumor cell invasion. Toxicol Sci.2010; 116:140-50.
6. Falagas ME, Grammatikos AP, Michalopoulos A. Potential of old-generation antibiotics to address current need for new antibiotics. Exp Rev Anti-inf Ther. 2008; 6 : 593- 600
7. Doull JL, Ahmed Z, Stuttard C,Vining LC. Isolation and characterization of *Streptomyces   venezuelae* mutants blocked in chloramphenicol biosynthesis. J Gen Microbiol. 1985 ;   131: 97–104
8. Vining LC, Stuttard C. Chloramphenicol. In Genetics and Biochemistry of Antibiotic Production, 1995; pp. 505–530. Edited by L. C. Vining and C. Stuttard Newton, M. A Butterworth-Heinemann.
9. Pestka S. Formation of transfer ribonucleic acid-ribosome complexes VII. Survey of the effect of antibiotics on N- acetylphenylalanyl puromycin formation: possible mechanism of chloramphenicol action. Arch Biochem Biophys. 1970; 36: 80-8
10. Michelinaki M, Mamos P, Coutsogeorgopoulos C, Kalpaxis DL. Aminoacyl and peptidyl analogs of chloramphenicol as slow binding inhibitors of ribosomal peptidyltransferase: a new approach for evaluating their potency. Mol Pharmacol. 1997; 51: 139- 46.
11. Vogel Z, Vogel T, Zamir A, Elson D. Correlation between the peptidyl transferase activity of the 50S ribosomal subunit and the ability of the subunit to interact with antibiotics. J Mol Biol. 1971; 60: 339- 46.
12. Bayfield MA, Dahlberg AE, Schulmeister U, Dorner S, Barta A. A conformational change in the ribosomal peptidyl transferase center upon active/inactive transition. Proc Natl Acad Sci. USA. 2001; 98: 10096-101.
13. Traversa G, Menniti-Ippolito F, Da Cas R, Mele A, Pulsoni A, Mandelli F. Drug use and acute leukemia.Pharm epidem drug Saf . 1998;7:113–23.
14. Yunis A. Drug-induced bone marrow aplasia. Rev Bras Pesqui Med Biol. 1978; 11: 287–295.
15. Kim HR, Shin MG, Kim MJ. Mitochondrial DNA aberrations of bone marrow cells from patients with aplastic anemia. J Kor Med Sci. 2008; 23:1062– 67.
16. Suazer CR, Ow EP .Chloramphenicol toxicity associated with severe cardiac dysfunction. Pediatr Cardiol. 1992; 13: 48 – 51.
17. Shukla P, Bansode FW, Singh RK. Chloramphenicol Toxicity: A ReviewJ Med Sci. 2011; 2 : 1313-16.
18. http://www.utdol.com/utd/login.do
19. Ambrose PJ. Clinical pharmacokinetics of chloramphenicol and chloramphenicol succinate. Clin Pharmacokin. 1984; 9: 222-38
20. Glazko AJ, Dill WA, Kinkel AW, Goulet JR, Holloway WJ, Buchanan RA. Absorption and excretion of parenteral doses of chloramphenicol sodium succinate in comparison with peroral doses of chloramphenicol. Clin Pharm Ther. 1977; 21: Abstract, p. 104.
21. DuPont HL, Hornick RB, Weiss CF, Snyder MJ, Woodward TE. Evaluation of chloramphenicol acid succinate therapy of induced typhoid fever and rocky mountain spotted fever. New Eng J Med. 1970; 282: 53–7.
22. Shann F, Linnemann V, Mackenzie A, Barker J, Gratten M, Crinis N.Absorption of chloramphenicol sodium succinate after intramuscular administration in children. New Eng J Med. 1985; 313: 410 – 4.
23. Slaughter RL, Pieper JA, Cerra B, Brodsky B, Koup JR. Chloramphenicol sodium succinate kinetics in critically ill patients. Clin Pharm Ther. 1980; 28: 69–77.
24. Acharya GP, Davis TM, Ho M, Harris S, Chataut C, Acharya S et al. Factors affecting the pharmacokinetics of parenteral chloramphenicol in enteric fever. J Antimicrob Chemother. 1997; 40: 91- 98.
25. Teow-Yee Ti, Monteiro EH, Lam S, Lee H. Chloramphenicol concentrations in sera of patients with typhoid fever being treated with oral or intravenous preparation. Antimicrob Agents Chemother. 1990; 34:1809 -11.
26. Tuomanen EI, Powell KR, Marks MI, Laferriere CI, Altmiller DH, Sack CM. Oral chloramphenicol in the treatment of *Haemophilus influenzae* meningitis. J Paediatr. 1981; 99: 968-74.
27. Yogev R, Davis AT. Oral chloramphenicol in the therapy of *Haemophilus influenzae* meningitis . Infection. 1981a; 9: 42- 44

28. Yogev R, Kolling WM, Williams T. Pharmacokinetic comparison of intravenous and oral chloramphenicol in patients with *Haemophilus influenzae* meningitis Pediatrics. 1981b; 67: pp. 656-60

29. Phongmanya S, Phetsouvanha R, Sisouphonea S. Trans of Royal Soc of Trop Med and hyg. 2005; 99: 451-58

30. Lasserrea R, Sangalangb RP Santiago L. Three-day treatment of typhoid fever with two different doses of ceftriaxone, compared to 14-day therapy with chloramphenicol: a randomized trial. J Antimcrob Chemther. 1991; 28: 765-72.

31. Gasem MH, Keuter M, Dolmans WMV,Ven-Jongekrijg JW, Djokomoeljanto R Jos WM et al. Persistence of *Salmonellae* in blood and bone marrow: Randomized controlled trial comparing ciprofloxacin and chloramphenicol treatments against enteric fever. Antimicrob Agents Chemother. 2003; 47 : 1727 - 31.

32. Aragones JV, Eriksen SP. Stable chloramphenicol solution for ocular infections. Am J Ophthalmol. 1968; 66: 104- 6.

33. Buckley SA. Survey of patients taking topical medication at their first presentation to eye casualty. Br Med J. 1990; 300: 1497-8.

34. Lam RF, Lai JSM, Ng JSK, Rao SK, Law RWK, Lam DSC. Topical chloramphenicol for eye infections. Honk Med J. 2002; 8 : 44 -47

35. Aragones JV, Eriksen SP. Stable chloramphenicol solution for ocular infections. Am J Ophthalmol. 1968; 66: 104-6.

36. Wallerstein RO, Condit PK, Casper CK, Brown JW, Morrison FR. State wide study of chloramphenicol therapy and fatal aplastic anaemia. J Am Med Ass. 1969; 208: 2045 –50.

37. Schaefer F, Bruttin O, Zografos L, Guex-Crosier Y.. Bacterial keratitis: a prospective clinical and microbiological study. Br J Ophthalmol. 2001; 85: 842– 47

38. Elton M. Conjunctivitis and chloramphenicol. The Pharm J. 2005; 274: 725-28

39. Bron AJ, Leber G, Rizk SN, Baig H,Elkington AR,Kirkby GR et al. Ofloxacin compared with chloramphenicol in the management of external ocular infection. Br J Ophthalmol.1991;75: 675-679

40. Darougar S, Viswalingam M, Treharne JD, Kinnison JR, Jones BR. Treatment of TRIC infection of the eye with rifampicin or chloramphenicol. Br J Ophthalmol.1977; 61: 255-59

41. Rose PW, Harnden A, Brueggemann AB, Perera R,Sheikh A, Crook D et al. Chloramphenicol treatment for acute infective conjunctivitis in children in primary care: a randomised double- blind placebo-controlled trial. Lancet. 2005; 366: 37 –43

42. Ramirez-Ortiz1 MA, Rodriguez-Almaraz1 M, Ochoa-DiazLopez H, Diaz-Prieto P, Rodriguez-Suárez RS.Randomised equivalency trial comparing 2.5% povidone-iodine eye drops and ophthalmic chloramphenicol for preventing neonatal conjunctivitis in a trachoma endemic area in southern Mexico. Br J Ophthalmol. 2007; 91: 1430-34.

43. Bourkiza R, Kaye S, Bunce C, Shankar J, Neal T, Tuft S. Initial treatment of *Pseudomonas aeruginosa* contact lens- associated keratitis with topical chloramphenicol, and effect on outcome. Br J Ophthalmol. 2013; 97: 429-32

44. McGhee CNJ, Anastas CN. Widespread use of topical chloramphenicol: is there justifiable concern regarding idiosyncratic aplastic anaemia? Br J Ophthalmol. 1996; 80:182-4.

45. Gardner FH. Chloramphenicol: a dangerous drug? Acta Haematol. 1991; 85:171-2.

46. Shackelford PG, Bobinski JE, Feigin, RD, Cherry JD. Therapy of *Haemophilus influenzae* meningitis reconsidered. New Eng J of Med. 1972;287: 634-38.

47. Feigin RD, Stechenberg BW, Chang MJ,Dunkle LM,Wong ML,Palkes H et al. Prospective evaluation of treatment of *Haemophilus influenzae* meningitis. The J of Ped. 1976; 88: 542 -8

48. Gleckman RA. Warning—chloramphenicol may be good for your health. Arch Int Med. 1975; l3S: 1125-26.

49. de Louvois, J. The bacteriology and chemotherapy of brain abscess. J Antimicrob Chemother .1978; A: 395-13

50. Lambert HP. Leading article. Problems with neonatal meningitis. J Antimicrob Chemother. 1977; 3: 381-82.

51. Ryder RW, Blake PA. Typhoid fever in the United States, 1975 and 1976. The J Infect Dis. 1979; 139: 124-26 .

52. Sharma KB, Bhat B, Pasricha M, Vaze S. Multiple antibiotic resistance among *Salmonellae* in India. J Antimicrob Chemother. 1979; 5: 15-21.

53. Schauf V, Green DC, Van der Stuyf L, Riff L. Chloramphenicol kills *Haemophilus influenzae* more rapidly than does ampicillin or cefamandole. Antimicrob Agents Chemother. 1983, 23 : 364-368

54. Pecoul B, Varaine F, Keita M, Soga G, Djibo A, Soula G, et al Long-acting chloramphenicol versus intravenous ampicillin for treatment of bacterial meningitis. Lancet. 1991; 338: 860-66.

55. Wali SS, Macfarlane JC, Weir WR, Cleland PG, Ball PAJ Hassan –King HG et al. Single injection treatment of meningococcal meningitis: long- acting chloramphenicol. Trans of Royal Soc of Trop Med and Hyg. 1974; 73: 689-701

56. Rahal JJ, Simberkoff MS .Bactericidal and bacteriostatic action of chloramphenicol against meningeal pathogens. Antimicrob Agents Chemother. 1979; 16: 13-18.

57. Whittle HC, Davidson B, Greenwood M, Warrell DA, Tomkins A, Tugwell P, et al. Trial of chloramphenicol for meningitis in Northern Savanna of Africa. Br Med J. 1973; 3: 379-381.

58.   Mackenzie AM, Chan FT. Combined action of chloramphenicol and ampicillin on chloramphenicol-resistant *Haemophilus influenzae*. Antimicrob Agents Chemother 1986; 29: 565- 69.

59.   Garvey RJP, Peter McMullin G. Meningitis due to beta lactamase producing type b *Haemophilus influenzae* resistant to chloramphenicol . Br Med J. 1983; 287:1183- 84

60.   Connor E, Melick C , Yogev R. In-vitro and in- vivo efficacy of ceftriaxone, moxalactam, and chloramphenicol against *Haemophilus influenzae* type b. J Antimicrob Chemother. 1982; 10 : 517-525

61.   Istre GR, Humphreys JT, Albrecht KD, Thornsberry C, Swenson JM, Hopkins RS. Chloramphenicol and penicillin resistance in pneumococci isolated from blood and cerebrospinal fluid: a prevalence study in metropolitan Denver. J Clin Microbiol. 1983, 3: 472-75.

62.   French L, Ling TKW, Davies DP, Leung DTY, et al. *Haemophilus influenzae* type b meningitis resistant to ampicillin and chloramphenicol. Arch of Dis in Childhood. 1986; 61: 691-707

63.   Solorzano GF, Munoz D. Haemophilus *influenzae* type b meningitis resistant to ampicillin and chloramphenicol . J Antimicrob Chemother. 1988; 22: 697-706

64.   Kim KSJ. Therapeutic efficacy of chloramphenicol, co-trimoxazole (trimethoprim/sulphamethoxazole), cefmenoxime and ceftriaxone in experimental bacteraemia and meningitis caused by ampicillin-resistant *Haemophilus influenzae* type b. Antimicrob Chemother.1988; 22 : 697-706

65.   Duke T, Michael A, Mokela D, Wal T, Reeder J.Chloramphenicol or ceftriaxone, or both, as treatment for meningitis in developing countries? Arch Dis Child. 2003; 88:536– 539

66.   Bhan MK, Bahl R, Bhatnagar S. Typhoid and paratyphoid fever. The Lancet. 2005; 366:749- 62.

67.   Balbi H. Chloramphenicol: A Review. Ped in Rev. 2004; 25: 284-88

68.   Renuka K, Sood S, Das BK, Kapil A. High-level ciprofloxacin resistance in Salmonella enterica serotype typhi in India. J Med Microbiol. 2005; 54:999-1000.

69.   Shirakawa T, Acharya B, Kinoshita S, Kumagai S, Gotoh A, Kawabata M. Decreased susceptibility to fluoroquinolones and *gyr A* gene mutation in the *Salmonella enterica serovar typhi* and *paratyphi* A isolated in Kathmandu, Nepal in 2003. Diagn Microbiol Infect Dis . 2006 ; 54 : 299-303

70.   Patel JC, Banker DD, Modi CJ. Chloramphenicol in typhoid fever a preliminary report of clinical trial in 6 cases. Br Med J. 1949; 22: 908-9

71.   Scioli G, Balestrieri GG. Comparison of ampicillin and chloramphenicol in treatment of typhoid fever. Postgrad Med J. 1964; 40: 87-91

72.   Rowe EJ, Threlfall LR, Ward A. Does chloramphenicol remain the drug of choice for typhoid? Epidem Inf. 1987; 98: 379-83

73.   Ellis ME, Moosa A , Hillier A.A review of typhoid fever in South African black children. Postgrad Med J 1990; 66: 1032- 36

74.   Islam A, Butler T, Alam NH. Treatment of typhoid fever with ceftriaxone for 5 days or chloramphenicol for 14 days: a randomized clinical trial. Antimicrob Agents Chemother .1993; 37 : 1572-75

75.   Gotuzzo E, Echevarria J, Carrillo C,Sanchez J,Grados P,Maguina C et al. Randomized comparison of aztreonam and chloramphenicol in treatment of typhoid fever Antimicrob Agents Chemother. 1994; 38: 558-62

76.   Jesudason MV, John R, John TJ. The concurrent prevalence of chloramphenicol- sensitive and multidrug-resistant *Salmonella typhi* in Vellore, S. India Epidemiol Infect 1996; 116: 225-27

77.   Butler T, Sridhar CB, Daga MK, Pathak K, Pandit RB, Khakria R et al. Treatment of typhoid fever with azithromycin versus chloramphenicol in a randomized multicentre trial in India. J Antimicrob Chemother. 1999; 44 : 243- 50

78.   Gasem MH, Keuter M, Dolmans WMV.Persistence of Salmonellae in blood and bone marrow: Randomized controlled trial comparing ciprofloxacin and chloramphenicol treatments against enteric fever. Antimicrob Agents Chemother. 2003; 47: 1727–31.

79.   Dimitrov T, Udo EE, Albaksami O, Kilani AA, Shehab EMR. Clinical and microbiological investigations of typhoid fever in an infectious disease hospital in Kuwait . J Med Microb. 2007; 56: 538–44

80.   Hammard OM, Hinfawy T, Omran D. Ceftriaxone versus chloramphenicol for treatment of acute typhoid fever. Life Sci J. 2011; 8: 100-5

81.   Harish BN, Menez GA. Preserving efficacy of chloramphenicol against typhoid Fever in a tertiary care hospital, India. Reg Health Forum. 2011; 16: 92-6

82.   Choudhary A, Gopalakrishnan R, Nambi S, Ramasubramaniam V, Ghaffur A, Thirunarayanan MA. Antibiotic susceptibility of Salmonella enterica serovar in a tertiary care hospital in Southern India. Ind J Med Res. 2013; 13: 800-2

83.   Anderson ES. The problem and implication of chloramphenicol resistance in the typhoid bacillus. J Hyg . 1975; 74:289-99.

84.   Paniker CKJ, Vilma KN. Transferable chloramphenicol resistance in *Salmonella typhi*. Nature. 1972;239:109 -10.

85.   Threlfall EJ, Rowe B, Ward LR. Occurrence and treatment of multi resistant *Salmonella typhi*. Public Health Laboratory Service Microbiology Digest. 1991; 8:56-9.

86.   Rowe B, Ward LR, Threlfall EJ. Multidrug-Resistant *Salmonella typhi*: A. Worldwide Epidemic Clin Inf Dis .1997; 24 : 106- 109

87. Sanghavi SK, Mane MP, Niphadkar KB. Multidrug resistance in Salmonella serotypes. Ind J Med Microbiol 1999; 17: 88-90.

88. Ackers ML, Puhr ND, Tauxe RV, Mintz ED. Laboratory based surveillance of *Salmonella* serotype *typhi* infections in the United States: antimicrobial resistance on the rise. J Am Med Ass. 2000; 283: 2668-2673.

89. Kumar R, Aneja KR, Roy P, Sharma M, Gupta R, Ram S.. Evaluation of minimum inhibitory concentration of quinolones and third generation cephalosporins to *Salmonella typhi* isolates. Ind J Med Sci. 2002; 56: 1-8.

90. Rahman M, Ahmad A, Shoma S. Decline in epidemic of multidrug resistant *Salmonella typhi* is not associated with increased incidence of Antibiotic susceptible strain in Bangladesh. Epidemiol Infect.2002; 129: 29-34.

91. Threlfall EJ, Ward LR. Decreased susceptibility to ciprofloxacin in *Salmonella enterica* serotype Typhi, United Kingdom. Emerg Infect Dis. 2001; 7: 448- 50.

92. Asna SM, Haq JA, Rahman, M. Nalidixic acid-resistant *Salmonella enterica serovar typhi* with decreased susceptibility to ciprofloxacin caused treatment failure: a report from Bangladesh. Jpn J Infect Dis. 2003; 56: 32-33.

93. Gay K, Robicsek A, Strahilevitz J, Park CH, Jacoby G, Barrett TJ, Medalla F, Chiller TM, Hooper DC. Plasmid-mediated quinolone resistance in non-Typhi serotypes of *Salmonella enterica*. Clin Infect Dis.2006; 43: 297-304.

94. Naiemi N Al, Zwart B, Rijnsburger MC, et al.Extended-Spectrum-Beta- lactamase production in a *Salmonella enterica* serotype typhi strain from the Philippines. J Clin Microbiol. 2008; 46: 2794 – 95.

95. Loannidis,AH. Chloramphenicol in treatment of acute respiratory infection. Lancet. 1957; 18:1157-1160

96. Kemmerich B, Borner K, Pennington JE.Comparative evaluation of enoxacin, ofloxacin, ampicillin, and chloramphenicol for treatment of experimental *Haemophilus influenzae* pneumonia. Antimicrob Agents Chemother. 1987, 20 : 417- 20

97. Howard AJ, Dunkin KT, Musser JM, Palmer SR. Epidemiology of *Haemophilus influenzae* type b invasive disease in Wales. Br Med J. 1991;303: 441- 45

98. Deivanayagam K, Nedunchelian TP, Ashok N, Mala D, Sheela S, Rathnam. R. Effectiveness of ampicillin and combination of penicillin and chloramphenicol in the treatment of pneumonias: randomized controlled trial. Ind Paed. 1996; 33: 813-16

99. Asghar R, Banajeh S, Egas J,Hibberd P, Iqbal I, Katep-Bawlya Met al. Chloramphenicol versus ampicillin plus gentamicin for community acquired very severe pneumonia among children aged 2-59 months in low resource settings: multicentre randomised controlled trial (SPEAR study) BMJ.2008; 336:80-4

100. Aspa J, Rajas O, Rodrı´guez de Castro F. Drug-Resistant pneumococcal Pneumonia: Clinical relevance and related factors. Clin Inf Dis. 2004; 38:787 –98

101. Campos J, Román F, Pérez-Vázquez M . Antibiotic resistance and clinical significance of *Haemophilus influenzae* type f. *J Antimicrob Chemother*. 2003; 52: 961– 66

102. Duke T. Neonatal pneumonia in developing countries Arch Dis Child Fetal Neonatal Ed. 2005; 90: F211– F219.

103. Hartmann C, Peter C, Hermann E . Successful treatment of vancomycin-resistant Enterococcus faecium ventriculitis with combined intravenous and intraventricular chloramphenicol in a newborn. J Med Microbiol 2010; 59: 1371–74.

104. Sood S, Kapil A, Das B, Jain Y, Kabra SK Re-emergence of chloramphenicol-sensitive Salmonella typhi. Lancet 1999; 353:1241-2.

105. Sowmiya M, Malathi J, Madhavan HN. Screening of ocular enterobacteriaceae isolates for presence of chromosomal blaNDM-1 and ESBL genes: a 2-year study at a tertiary eye care center . Invest Ophthalmol Vis Sci. 2012; 53:5251-7