“In all things there is a poison, and there is nothing without a poison. It depends only upon the dose whether a poison is a poison or not”.

Paracelsus (1490 – 1541)

CHAPTER 1

1. INTRODUCTION TO ANTIBIOTICS

1.1 DEVELOPMENT OF ANTIBIOTICS

Antibiotics have been called the single most important therapeutic discovery in the history of medicine. An interesting feature of their historic discovery is that they occurred within the lifetime of many of the population living today. It would appear, however, that antibiotics were used even before their discovery. Ancient writings report the application of cloths impregnated with natural substances and other forms of organic matter onto wounds to help them heal. Anthropologists have also unearthed traces of tetracycline in thousand-year old Nubian mummies. The Nubian nation survived from ancient times to the 14th century and some scientists suggest that this might be partially linked to the presence and use of antibiotics such as the tetracyclines [Levy, 1984].

Modern antimicrobial chemotherapy developed in three eras. The first, from 1600 to 1900, involved the use of Cinchona bark in the treatment of malaria. Quinine, the active ingredient, was isolated only later in the 1820s. The synthetic era, which marked the development of various dyes to treat bacterial infections, arrived around the beginning of the 1900s. The first was pyocyanase, a blue pigment produced by *Pseudomonas aeruginosa*, which reduced the growth of other bacteria *in vitro*. When used *in vivo*, it was found to be unstable and toxic. Similar problems occurred with Salvarsan, used in the treatment of syphilis, and Prontosil, active against streptococci *in vitro* [Edwards, 1980].

Possibly the most important event in the discovery of antibiotics occurred around 1940 and began the era of the antibiotics. Although Alexander Fleming discovered penicillin in 1929, it was only recognised for its potential and potency by Florey and his team in 1940. These so-called “miracle drugs” were so scarce in the beginning that penicillin was often re-extracted from the urine for subsequent dosage. Early in the antibiotic era, this valuable agent was freely available to the public in over-the-counter preparations including throat lozenges, nasal ointments and even cosmetic creams. By 1955 most countries restricted its use to prescription only but widespread usage had already occurred, which led to widespread resistance. An answer to this resistance came in the form of methicillin in the early 1960s and this semi-synthetic penicillin was not vulnerable to the bacterial enzymes that degraded penicillin. Other derivatives soon followed but this advance soon revealed the growing pattern of resistance to all available antibiotics [Levy, 1984].

Today the biggest killers in western cultures include cardiovascular disease, neoplasms, nervous system ailments, and not surprisingly, microbial infections. In indigenous people, whose lifestyles differ considerably from western society, other ailments such as diarrhoea, maternity complications and inflammation are regarded as more of a threat to survival [Cox, 1994]. The role that bacterial diseases play in these cultures should not be underestimated since studies performed among inhabitants of West Africa have shown the main endemic bacterial diseases include leprosy, tuberculosis, bacillary dysentery, enteric fevers, undulant fevers and occasionally cholera [Oliver-Bever, 1986].

It is only since the 1940s that we have seen the rapid development and production of antibiotics and the quest for new antibiotics continues but at a slower pace. New drug discoveries often come up with a derivative member of a family already described but more initiatives to find truly novel antibiotics are required and are the current goals for the new millennium [Levy, 1984].

1.2 ANTIBIOTIC RESISTANCE

Drug abuse usually conjures up images of secret meetings in dark allies, gangs and “spaced out” teenagers lying on the floors of clubs. The use of heroin, cocaine, marijuana and even the dependency of stressed-out executives and housewives on anti-anxiety drugs or antidepressants is considered to be substance abuse. Recently a new way of detecting erythropoeitin, used by athletes for performance enhancement, has been brought into action to disqualify those abusing it.

Most of us seem to agree that the world is relying far too much on drugs to either keep us awake or help us to sleep, suppress or stimulate the appetite, control depression and stress and generally make our lives “more bearable”. Few of us would, however, consider the overuse of antibiotics as abuse. Yet the abuse of these compounds could spell more devastating worldwide health consequences to both humans and animals than the accumulated abuse of all the world’s narcotics put together [King, 2000].

Almost since the beginning of the antibiotic era, bacterial resistance has been seen as the major obstacle to successful treatment. Hardly any group of antibiotics has been introduced to which some bacterium has not developed resistance. Resistance was often minimised as a problem simply because the problem was not known or recognised. At the end of the 1960s the Surgeon General of the United States stated that “we could close the book on infectious diseases.” At the time he uttered these words the emergence of resistance did not seem to affect therapeutic options and although *S. aureus* had become resistant to benzylpenicillin and showing resistance to methicillin, it remained sensitive to gentamicin and infections could therefore still be treated [Amyes, 2000].

At the start of a new century, things look very different. Already at least three bacterial species capable of causing life-threatening illness (*Enterococcus faecalis, Mycobacterium tuberculosis* and *Pseudomonas aeruginosa*) are already known to be resistant to every one of the over 100 antibiotics available except for vancomycin. Vancomycin is the antibiotic of last resort for treatment of resistant infections and within the past year scientists have found strains of *Streptococcus pneumoniae* and *Staphylococcus aureus* to be resistant to this antibiotic. With the advent of these reports and new cases emerging continuously, an ingrown toenail, sore throat or a dirty cut could spell death.

Why is this happening? Well firstly we tend to view all bacteria as "the enemy" to be quickly dealt the deathblow with antiseptics, disinfectants and antibiotics. In human medicine alone, the US Centre for Disease Control and Prevention estimates that approximately one-third of the 150 million prescriptions for antibiotics written each year were unneeded [http://www.agric.gov., 2000].

Although antibiotic discovery has been exponential since the 1940s, no new clinically useful drugs were discovered after 1961 and almost all the drugs that have been launched since the 1960s are modifications of the antibiotics we already have. The introduction of organ transplantation in the late 1960s prompted the massive increase in antibiotic use, which was accompanied by an increase of methicillin resistance in staphylococci and vancomycin resistance in enterococci [Amyes, 2000].

Recent reports have also shown a marked increase of antibiotic resistance of food-poisoning bacteria due to non-rational and excessive uses of antibiotics as therapeutic agents or as growth promoter in livestock. Another aspect of resistance lies in the use of antibiotic resistant genes as selection markers in genetically modified organisms (GMOs). The main safety factor concern is the release of the resistant genes to sensitive organisms when these GMOs are introduced into the environment [http://www.biosafety, 1999].

1.2.1 Battlegrounds

There are two main zones of combat today, the hospital and the community, which are neither discrete nor separate. Patients are being discharged from the hospital into the community and those with communal infections are being hospitalised. The ease of travel means the transport of resistant organisms into foreign lands, making it a global problem. As a result of this increasing and dangerous problem, the Centre of Disease Control (CDC), World Health Organisation (WHO) and local public health groups have initiated various surveillance programs. Some of these programs have been monitoring resistance for decades and many pharmaceutical companies have supported national and international surveillance programs. Despite all of these efforts it is evident that a more deliberate attention is required and *grass-roots* methods employed [Operation Resistance, 2000].

1.2.2 Battle lines

Enterobacteriaceae

One of the largest groups of bacteria is the Enterobacteriaceae, presently comprising over 120 species. These are grouped in genera of which the most infamous include *Klebsiella, Enterobacter* and *Serratia* (K.E.S.). Also included are *Escherichia, Proteus, Salmonella, Shigella* and *Yersinia*.

These members are found in many different locations and have been associated with virtually every type of infection covering abscesses, pneumonia, meningitis, septicaemia and other site-specific infections. Collectively these organisms are responsible for one in six nosocomial infections. Internationally these organisms have caused significant hospital outbreaks in France, Germany, Australia, UK, Greece, Tunisia and the USA [Operation Resistance, 2000].

The production of simple β-lactamases by the Enterobacteriaceae has been recognised for years and since the 1970s, resistance to the current β-lactams, trimethoprim-sulphamethoxazole (TMP/SMX) and often the aminoglycosides, was causing outbreaks of nosocomial infections. In the 1980's, broader spectrum agents were developed, which included the second- and third-generation cephalosporins and the aminopenicillins. Soon it became evident that these organisms evolved their own β-lactamases in response to environmental pressure and these enzymes are known as Extended Spectrum β-Lactamases (ESBLs). These enzymes soon inactivated agents such as cefoxitin, cefotetan and more recently, the carbapenems. These ESBLs destroy most second- and third generation cephalosporins but also have activity against potentiating agents such as clavulanic acid, tazobactam and sulbactam. This resistance may also accompany resistance to other classes of antibiotics. Table 1.1 shows the susceptibility to a range of commonly prescribed hospital antibiotics.

Table 1.1: Antimicrobial Susceptibility (%) of Selected Nosocomial Pathogens [Adapted from Operation Resistance, 2000]

Antimicrobial agent	*E. coli*	*K. pneumoniae*	*E. aerogenes*	*E. cloaceae*
Cefuroxime	95	91	77	59
Ceftazidime	96	92	81	77
Cefotaxime	98	89	77	67
Ceftriaxone	97	96	75	77
Pipercillin-tazobactam	95	87	74	77
Imipenem	97	98	95	97
Gentamicin	97	95	97	96
Ciprofloxacin	96	88	88	93

Examination of data from the NNIS showed a linear increase in resistance of *Enterobacter* species and *Klebsiella pneumoniae* to ceftazidime over the periods 1987-1989 to 1990-1991 respectively:

K. pneumoniae 1.5% - 3.6%

Enterobacter sp. 32.9% - 38.6%

This ability to cause new mutations and therefore a broader range of β-lactam inactivating enzymes provide these organisms with a protective shield from beneath which they can cause significant morbidity and mortality.

Respiratory Tract Infections

Approximately 63% of antibiotic prescriptions are written for respiratory tract infections (RTIs) [Operation Resistance, 2000] and these infections account for nearly half of all antibiotics used in intensive care units [Singh, 2000]. Of these infections, the one most feared is that caused by *Streptococcus pneumoniae*. Globally each year at least one million people die due to *S. pneumoniae* infections and its increasing resistance to antibiotics is frightening. Already in 1945 resistant mutants were created in the laboratory setting and since 1967 it has travelled the globe a remarkable number of times. During these travels, MICs to penicillin have increased as well as resistance to other antimicrobial drugs including the macrolides, TMP/SMX, tetracyclines and chloramphenicol. One survey in the USA shows that penicillin resistance is present in approximately 35% of all community-derived isolates of *S. pneumonia* (see Figure 1.1) [Operation Resistance, 2000], while another reported an increase of 45% [Gotfried, 2000]. Slightly lower levels of resistance to other antimicrobials are also demonstrated with the exception of the newer quinolones where very few resistant strains have been seen.

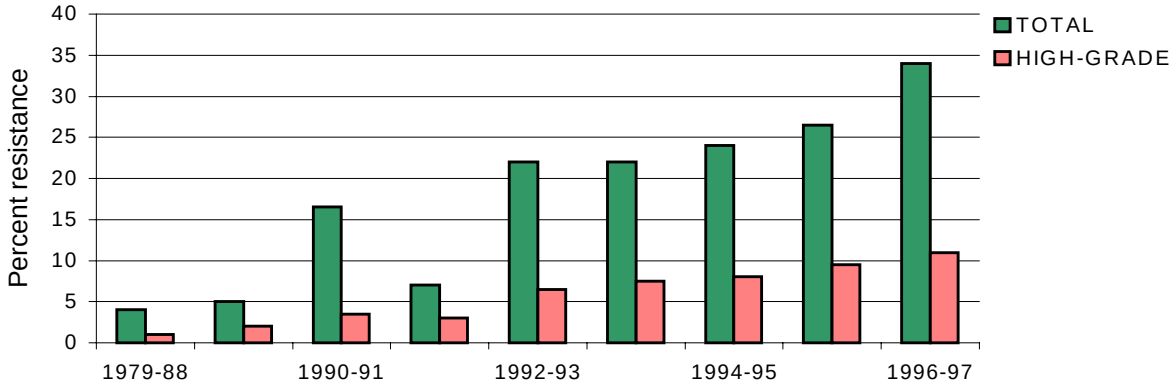

Figure 1.1: Percentage of the total and high-grade resistance of *S. pneumoniae* to penicillin [adapted from Operation Resistance, 2000]

Other respiratory pathogens include *Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus* and *Streptococcus pyogenes*. Since 1970 the incidence of β-lactamase producing *H. influenzae* strains have risen so that today over a third are resistant to amoxicillin. Only the quinolones are currently impervious to its resistance [Operation Resistance, 2000]. A surveillance study of patients with respiratory infections in Saudi Arabia showed a 13.2% resistance of *H. influenzae* to ampicillin, 7% to tetracycline, 5.4% to chloramphenicol, 3.9% to roxithromycin and 1.6% to amoxicillin/clavulanic acid [Abdel-Rahman, 1999].

Staphylococcus aureus is also a leading cause of nosocomial infections and is almost always resistant to β-lactams, macrolides and tetracyclines, leaving few alternatives [Operation Resistance, 2000].

According to the SENTRY Antimicrobial Surveillance Program (1998) the four most frequently isolated organisms isolated from the lower respiratory tract of patients hospitalised with pneumonia were *P. aeruginosa* (26.8%), *Staphylococcus aureus* (24.0%), *Klebsiella pneumoniae* (12.1%) and *Acinetobacter* spp. (10.5%). Of the more than 40 antimicrobial agents tested, amikacin (77.5% susceptible) was the most active drug tested against *P. aeruginosa*. Only meropenem (78.3%) and imipenem (81.3%) possessed susceptibility rates greater than 50% against the *Acinetobacter* spp. isolates. Resistance to oxicillin by *S. aureus* was nearly 50% and several clusters of multiple resistant organisms were observed. These results indicate the rapid rates of resistance among respiratory tract pathogens and the concern of multiple resistant strains in several medical centres [Lewis, 2000]. Figure 1.2 shows the percentage resistance of respiratory tract isolates versus non-respiratory tract isolates over a period of eight years. Respiratory tract isolates became notably more resistant to imipenem, ceftazidime, cefotaxime, ticarcillin/clavulanic acid and piperacillin than were non-respiratory isolates [Flournoy, 2000].

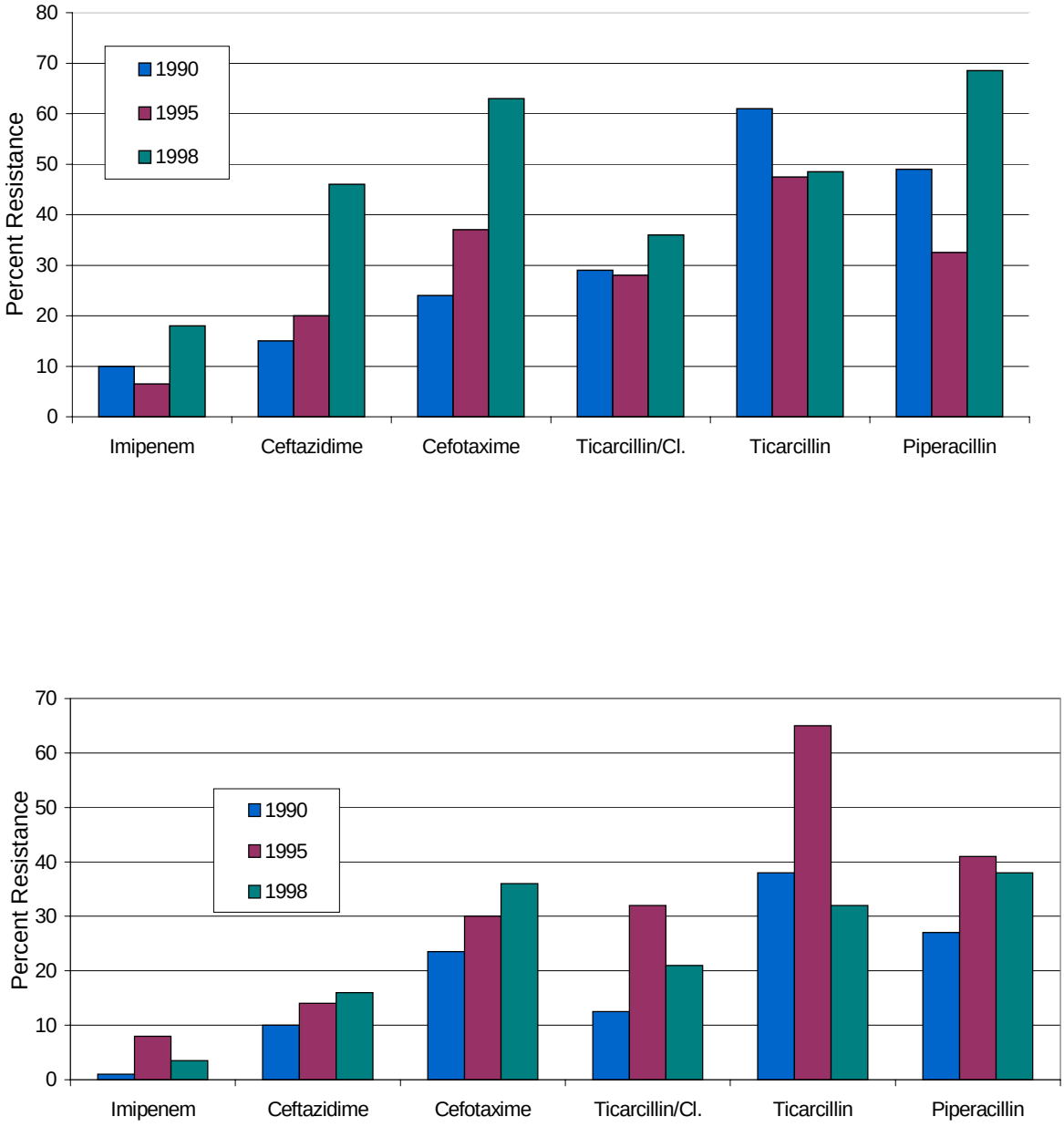

Figure 1.2: The increasing resistance of respiratory initial isolates (top graph) and non-respiratory isolates (bottom graph) between 1990 and 1998 to different antibiotics. (Ticarcillin/Cl. is the combination of Ticarcillin with clavulanate) [Adapted from Flournoy, 2000].

Urinary tract infections (UTIs)

The cost and treatment of this infection costs at least $1 billion annually in the USA. Table 1.2 shows the pathogens responsible for UTIs and susceptibility rates of various antimicrobials.

Table 1.2: Susceptibility rates of various antimicrobials in acute cystitis in percentage [Adapted from Operation Resistance, 2000]

Pathogen	Incidence	Ampicillin	TMP/SMX	Nitrofurantoin	Ciprofloxacin
E. coli	86	74	91	99	99.8
S. saprophyticus	4	0	92	100	?
Proteus spp	3	92	95	0	100
Klebsiella spp	3	2	91	46	100
Enterobacter spp	1.4	8	98	48	100
Citrobacter spp	0.8	12	97	82	100

As there has been only one novel class of antimicrobial agents in the last 25 years, it is essential that something be done to either prescribe the existing drugs more rationally, to try to minimise infection or to find novel antimicrobial agents [Operation Resistance, 2000].

1.3 QUANTITATIVE ASPECTS

Clinically, *in vitro* antimicrobial susceptibility tests are useful as a guide for determining antimicrobial chemotherapy whenever the susceptibility of a pathogen is unpredictable or when an infection has not responded to therapy that otherwise appears appropriate [NCCLS, 1991].

Minimum inhibitory concentration (MIC) is defined as the minimum concentration required to inhibit 50% of a bacterial population. MIC however, does not represent an absolute value and the “true” MIC is somewhere between the lowest

test concentration that inhibits the organism growth ("read" MIC) and the next lower test concentration. Even under the best of controlled conditions a dilution test may not yield the same end-point each time it is repeated but gives an indication of the concentration of an antimicrobial agent that must reach the site of infection to inhibit the infecting organism. Table 1.3 gives an indication of MIC values of a selected range of antibiotics against four reference strains.

Table 1.3: Acceptable quality control ranges of MICs (μg/ml) for reference strains (Adapted from NCCLS, 1991)

Antibiotic	***S. aureus*** **ATCC 29213**	***E. faecalis*** **ATCC 29212**	***E. coli*** **ATCC 25922**	***P. aeruginosa*** **ATCC 27853**
Amikacin	1-4	64-256	0.5-4	0.5-8
Azithromycin	0.28-1	-	-	-
Cefaclor	1-4	>32	1-4	-
Cefazolin	0.25-1	>16	1-4	-
Cefuroxime	0.5-2	-	2-8	-
Chloramphenicol	2-8	4-16	2-8	-
Clarithromycin	0.12-0.5	-	-	-
Erythromycin	0.12-0.5	1-4	-	-
Gentamicin	0.12-1	4-16	0.25-1	0.25-4
Lomefloxacin	0.25-2	2-8	0.03-0.12	1-4
Methicillin	0.5-2	>16	-	-
Ofloxacin	0.12-1	1-4	0.015-0.12	1-8
Penicillin G	0.25-1	1-4	-	-
Teicoplanin	0.25-1	0.06-0.25	-	-
Tetracycline	0.25-1	8-32	1-4	8-32
Vancomycin	0.5-2	1-4	-	-
Trimethoprim	1-4	≤1	0.5-2	>64

1.4 CONCLUSION

In a crisis such as this where we are faced with the desperately growing need for novel antimicrobial compounds in the fight against resistance, it seems logical to turn towards Mother Nature for answers. Secrets to her success have been discovered over many centuries within various sources and a survey conducted in 1967 calculated that over 58% of all antibiotics are produced by Actinomycetes, 18% by fungi, 12% by higher plants, 9% by bacteria and the remaining 3% by algae, litchens and animals [Edwards, 1980]. Although these antibiotics are now synthesised in laboratories, they were originally discovered inside living organisms. For this very reason it became the aim of this study to review natural resources, in particular *Combretum erythrophyllum* used by traditional healers in their treatment of infection, with the hope of isolating and identifying the compounds responsible for its activity. This is discussed in further detail in Chapters 2 and 3.