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Clostridium: Pathogenic Roles, Industrial Uses and Medicinal Prospects of Natural Products as Ameliorative Agents against Pathogenic Species

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Abstract

The genus *Clostridium* is made up of species that cause disease to both human beings and animals, with zoonotic species/strains playing critical roles in disease dynamics. Clostridial organisms posses pathogenic, therapeutic and industrial uses. Pathogenic clostridia cause lethal or life threatening infections that must be treated, while the industrial clostridia produce bio-fuels, to serve as alternative energy sources in the face of very high global prices of conventional fuels. Genetic engineering is currently developing novel strains by replacing or altering the gene configuration of pathogenic strains (gene knockout/down) to convert them to solvenogenic and non pathogenic strains for industrial uses. Therapeutic clostridia serve as vehicles for treatment of diseases, especially solid tumors. Because of the global problem of antibiotic resistance, which is a survival strategy by microbial pathogens, some of the well known chemotherapeutic protocols have failed and there is the need to evolve new treatment approaches, using novel agents that have superior mechanisms of action, compared to conventional antibiotics that are becoming inconsequential because of resistance. It is believed that natural products may be effective alternatives to resolving this puzzle, since they are cheaper, readily available and possibly effective against clostridial species. In this review article, the authors discussed some of the life threatening and solvenogenic clostridia and listed some natural products that may possibly be employed as drug targets for ameliorating the problem of resistance in the future, if their active principles are thoroughly researched. It is concluded that medical and veterinary research should be re-jigged to evolve active principles from natural products that are not so easily surmounted by the surge in drug resistant strains.

Keywords: *Clostridium*; Industrial Uses; Medicinal Prospects; Natural Products; Ameliorative Agents; Pathogenic Species..

1. Introduction

The genus *Clostridium* consists of over 100 species, ranking second in size next to Streptomyces (Dong *et al.*, 2010). Many *Clostridium* species are known to cause disease to human beings. These include neurotoxigenic clostridia (*C. botulinum* and *C. tetani*) (Weingart *et al.*, 2010), clostridia involved in gas gangrene and necrotizing infections (*C. perfringens, C. sordellii* and *C. septicum*) (Hatheway, 1990; Aldape *et al.*, 2006), and the enteropathogenic *C. difficile* (Twine *et al.*, 2009). *Clostridium chauvoei*, which has a strong phylogenetic relationship with *C. septicum*, a human pathogen, has a long history of veterinary importance. It was believed for a very long time that this species was exclusively a veterinary pathogen, only associated with blackleg in ruminants (Useh *et al*., 2006a). Recent reports of the disease in human beings have placed the pathogen on the list of very important lethal disease agents of human beings (Nagano *et al.*, 2008; Weatherhead and Tweardy, 2011). Indeed zoonotic strains of the *Clostridium* spp pose a great threat to community health in endemic areas. The aforementioned notwithstanding, genetic engineering of pathogenic strains is beginning to suggest that members of the genus could also serve therapeutic purposes. In tumor managements, the use of viral vehicles in gene therapy could be successful or not, depending on the delivery systems employed. There are many tumors in

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which this procedure has not been successful. Thus, there is intensive on-going research in so many expert laboratories to develop alternative management approaches (Minton, 2003). On the other hand, some *Clostridium* species are of great industrial importance. For example, *C. thermocellum* can produce ethanol from lignocellulosic waste at high temperature, while *C. acetobutylicum* and *C. beijerinckii* produce solvents (acetone, butanol, and ethanol) by utilizing a variety of substrates from monosaccharides to polysaccharides (Ezeji *et al.,* 2007; Otte *et al.,* 2009; Ezeji *et al*., 2010; Jia *et al*., 2011).

2. 2. Pathogenic Clostridia

2.1. Clostridium Difficile

Clostridium difficile is the most common cause of infectious diarrhoea in hospitalized patients in the industrialized world (Johnson and Gerding, 1998). With symptoms ranging from self-limited diarrhoea to life threatening fulminant colitis, *C. difficile* infection (CDI) has affected hundreds of thousands of patients worldwide and substantially burdens health care resources (Kyne *et al.,* 2002). In particular, CDI has caused increased patient morbidity and mortality in hospitals throughout the world since 2003, when outbreaks with increased disease incidence and severity first emerged (Forgetta *et al*., 2011), and from 2004 to 2007 contributed to almost 1,000 deaths in the province of Quebec, Canada (Gilca *et al*., 2008 cited by Gilca *et al*., 2011). Investigations of these and other outbreaks across Canada, the United States, and Western Europe led to the recognition of a severe-disease associated (SDA) strain predominantly responsible for this epidemic (Kuijper *et al.,* 2006). This strain has been classified as North American pulse field type 1 (NAP1), ribotype 027, toxinotype III, or restriction-endonuclease type BI. Outbreaks have also been associated with other SDA strains, such as the NAP7/toxinotype V/ribotype 078 (NAP7) strain found in cases of human and animal disease (Goohuis *et al.,* 2008; Mulvey *et al.,* 2010) and multiple toxin A-negative B-positive (A-B+) pulsotypes and ribotypes responsible for CDI outbreaks in Ireland, the United Kingdom, the United States, and Canada (Al-Barrack *et al*., 1999; Stabler *et al*., 2006). *Clostridium difficile* is the leading cause of health care–associated infectious diarrhoea. After exposure to *C. difficile*, some patients remain asymptomatic, whereas others have illness ranging from mild diarrhoea to fulminant colitis (Johnson and Gerding, 1998). Outbreaks of *C. difficile* infection in North America and Europe have been attributed to the emergence of an epidemic strain (North American pulsed-field gel electrophoresis [PFGE] type 1 [NAP1]) (McDonald, 2005).

Clostridium difficile is the most commonly identified bacterial cause of HIV-related and all nosocomial diarrhoea (Bartlett, 2007). Whereas outbreaks in North American and European hospitals have led to increased *C. difficile* infection (CDI) vigilance in developed countries, data on its significance for developing countries is scarce (Warny *et al*., 2005). This information gap is significant since antibiotic pre-exposure, an important CDI risk factor, is high in many developing countries due to unregulated access (Sosa, 2005). Recent data suggest CDI is under-recognized in Latin America (Legaria *et al.,* 2003). Data from sub-Saharan Africa is sparse. Despite liberal, unregulated antibiotic use, developing countries do not have data about *C. difficile* infection. Nigeria, Africa's most populous country, has the third highest absolute HIV burden worldwide. Over-the-counter antibiotic access is widespread in Nigeria and nosocomial diarrhoea incidence is high (Nwokediuko *et al.,* 2002). However, patients are not tested for CDI due to presumptions of low prevalence and technical incapacity. Result from the study by this group showed significant CDI in patients with HIV infections in a 5 star Hospital in Nigeria.

C difficile has been reported as a pathogen of animals, affecting dogs, cats, horses and pigs (Weese *et al*., 2001a,b; Arroyo *et al*., 2005; Keel *et al*., 2007; Keessen *et al*., 2010, 2011). In one study, some authors reported *C. difficile* colonization in dogs and cats in a longitudinal manner, indicating that *C. difficile* is commonly albeit sporadically found in the faeces of healthy pets (Weese *et al*., 2010). Another study in the Netherlands showed high *C. difficile* prevalence in dogs, cats, horses, pigs, poultry and calves. Twenty-two different PCR ribotypes were identified and all *C. difficile* isolates from pigs, cattle and poultry were toxinogenic, whereas the majority of isolates from pet animals consisted of non-toxinogenic PCR ribotypes 010 and 039. Ribotype 012 was most prevalent in cattle and ribotype 078 in pigs. No predominant ribotypes were present in horse and poultry samples. Overall, PCR ribotypes 012, 014 and 078 were the most frequently recovered toxinogenic ribotypes from animal samples. Comparison with human isolates from the Dutch Reference Laboratory for *C. difficile* at Leiden University Medical Centre (LUMC) showed that these types were also recovered from human hospitalized patients in 2009/2010, encompassing 0.8%, 11.4% and 9.8% of all isolates, respectively. Application of multiple locus variable-number tandem-repeat analysis indicated a genotypic relation of animal and human ribotype 078 strains, but a clear genotypic distinction for ribotypes 012 and 014. Contrary to PCR ribotype 078, significant genetic differences were observed between animal and human PCR ribotype 012 and 014 isolates. The authors concluded that toxinogenic *C. difficile* PCR ribotypes found in animals correspond to PCR ribotypes associated with human disease in hospitalized patients in the Netherlands (Koene *et al*., 2011).

It is believed that *C. difficile* is the most important cause of outbreaks of neonatal diarrhoea in pig husbandry (Songer and Uzal, 2005). Infection of neonatal piglets occurs by environmental transmission (Hopman *et al.,* 2010). The bacteria have also been detected in air samples, but the role of aerial dissemination in the transmission of *C. difficile* to piglets has yet to be conclusively elucidated (Hopman *et al.,* 2010). *C. difficile* has also been reported in retail meat in Sweden (von Abacron *et al.,* 2009), broiler chickens sold at market places in Zimbabwe (Simango and Mwakurudwa, 2008), chicken in retail poultry meat

in the United States of America (Songer *et al*., 2009; Harvey *et al*., 2011a,b), retail vegetables in Canada (Al-Salif and Brazier, 1996; Bakri, 2009; Metcalf *et al*., 2010) with a zero prevalence in passerine birds in Europe (Bandelj *et al*., 2011). The meaning of all these reports is that *Clostridium* as an organism is set to colonize the entire environment to create havoc to public health.

In the light of the ongoing uncertainty about the zoonotic potential of *C. difficile* and the emergence of hypervirulent strains, research on the possible routes of transmission from animals to human beings and between animals is becoming increasingly important. There is still little knowledge on possible transmission routes from animals to human beings (Jhung *et al.,* 2008). Possible routes other than meat consumption (direct and indirect contact) with animals, have been speculated (Weese, 2010). It is increasingly recognized that aerial dissemination of bacterial elements can be the main route of zoonotic transmission (Kuske, 2006; Keessen *et al*., 2011).

2.2. Clostridium Chauvoei

Blackleg in ruminants has been reviewed (Useh *et al.,* 2006a). It is a fatal disease of cattle and sheep mainly (but has also been reported in deer and other animal species), caused by *C. chauvoei*, and was first reported in 1870 (Armstrong and McNamee, 1950). Other synonyms of the disease are: black quarter, emphysematous gangrene, symptomatic anthrax, quarter ill (Merchant and Barner, 1964), gangrenous myositis (Williams and Andrews, 1992), clostridial myositis of ruminants (Williams, 1977) and clostridial myocarditis (blackleg of the heart) (Uzal *et al*., 2003). *C. chauvoei* was named after Professor J. A. B. Chauveau, a French bacteriologist (Cato *et al*., 1986), and 23 reference strains of the bacterium have been described (Holderman *et al.,* 1977). In Nigeria, Jakari, Vom and K76 strains have been incriminated as the aetiologic agents of the disease (Bagadi, 1977), while NCTC 8076 and 8361 (Heurmann *et al.,* 1991), strain 49 (Singh *et al*., 1993), strain NC 08596 (Heurmann *et al.,* 1991), strains ch.16 and ch.22, strain Tukyu and strain Awasa (Mhoma *et al.,* 1995) have been reported to cause blackleg in UK, India and other parts of Asia, Germany, other parts of Europe and the Americas, Mozambique, Tanzania and other parts of east Africa and Ethiopia, respectively. These strains are also responsible for the disease in both the natural and accidental hosts (Radostits *et al.,* 2000).

2.3. Clostridium Botulinum

Botulinum neurotoxins are 150-kDa endopeptidase toxins that are produced by C. *botulinum, C. butyricum* and *C. baratii* (CDC, 1998). These neurotoxins are considered the most toxic substances known (Lamanna, 1959) and have been designated as category A biological threat agents (Rotz *et al.,* 2002). Botulism, a serious neuroparalytic and sometimes fatal illness, caused by potent neurotoxins produced by the gram-positive, anaerobic, spore-forming bacterium *C. botulinum*, affects both animals and human beings. Outbreaks have been reported following consumption of unsafe canned vegetables (Date *et al*., 2011). Rodloff and Krüger (2012) reviewed the occurrence of visceral botulism in farmers. Seven types of botulinum toxins are known (A through G), of which types A, B, E, and F cause virtually all cases of human botulism (CDC, 1998). Botulism is characterized by rapidly progressive cranial neuropathy and symmetric descending flaccid paralysis, which may progress to respiratory arrest requiring mechanical ventilation and intensive supportive care in 60% of patients and clinical recovery takes several weeks to months (Shapiro *et al.,* 1998). Botulism neurotoxin derivative has been engineered to allow self activation (Masuyer *et al.,* 2011).

2.4. Clostridium Septicum

The organism is the causative agent of muscular gangrene syndrome with clinical features that overlap with those of other clostridial diseases and acute/hyperacute syndromes seen with other diseases such as anthrax (Fasanella *et al.,* 2010). *C. septicum* and *C. chauvoei* have a close phylogenetic relationship that made science unable to distinguish the two organisms convincingly for a very long time (Nagano *et al*., 2008). Traditional microbiological methods are unable to distinguish the two microbial species. Molecular diagnostics, based on real time PCR with primers designed according to sporulation, 16s rRNA and triose pentose isomerase gene sequences have been reported (Lange *et al*., 2010; Ham *et al*., 2010; Garofolo *et al*., 2011). Soft tissues are usually affected in the pathology of the disease with an accompanying necrotizing infection (Gnerlich *et al.,* 2011; Kiel *et al*., 2011).

2.5. Clostridium Perfringens

C. perfringens is a Gram-positive rod, spore-forming, anaerobic bacterium, which is highly associated with a wide array of gastrointestinal (GI) and histotoxic diseases in both humans and animals (McClane, 2007; Amimoto *et al.,* 2007; Keyburn *et al.,* 2008). The most common cause of *C. perfringens*-associated food poisoning in humans is the consumption of *C. perfringens* vegetative cells followed by sporulation in the gut (Paredes-Sabja and Sarker, 2009). The early steps in the development of *C. perfringens*-associated gas gangrene and other non-food-borne GI illnesses, such as antibiotic-associated diarrhoea, occur when *C. perfringens* spores ubiquitously found in the environment come in contact with the host, where spores undergo germination followed by outgrowth, cell proliferation and toxin secretion (Paredes-Sabja and Sarker, 2012).

3. Non-Pathogenic Clostridia

The biofuel producing clostridia are very important as they are nonpathogenic and produce biogenic fuels for industrial use (Köpke *et al.,* 2011). For instance, 2,3-Butanediol (2,3BD) is a commodity chemical usually produced from oil. It can be used as a precursor in the manufacture of a range of chemical products, including the solvents methyl ethyl ketone (MEK), gamma-butyrolactone (GBL), and 1,3-butadiene. Commercially, the key downstream products of 2,3BD have a potential

global market of around 32 million tons per annum, valued at approximately $43 billion in sales (Xiu and Zeng, 2008). Three (3) acetogenic members of the *Clostridium* genus (*C. autoethanogenum, C. ljungdahlii*, and *C. ragsdalei*) have been reported to produce 2,3 BD using gases (carbon monoxide [CO] or hydrogen [H_2] and CO) as the source of carbon and energy. 2,3-butanediol (2,3BD) is a high-value chemical usually produced petrochemically but can also be synthesized by some bacteria. To date, the best microbial 2,3BD production rates have been observed using pathogenic bacteria in fermentation systems that depend on sugars as the carbon and energy sources for product synthesis. Homologues of the genes involved in the requisite pathway have been identified (Köpke *et al*., 2011). In the report, a gene expression study demonstrated a correlation between mRNA accumulation from 2,3BD biosynthetic genes and the onset of 2,3BD production, while a broader expression study of Wood-Ljungdahl pathway genes has been shown to provide a transcription-level view of one of the oldest existing biochemical pathways. Genetic engineering is in its top form to develop new stains of clostridial organisms with non-pathogenic factors and great industrial uses (Zhang *et al*., 2012).

Acetone-butanone-ethanol (ABE) fermentation involves the production of these via fermentation. This is re-invigorated by the high and ever rising cost of crude and the desire for alternative energy sources to cut cost. As a result of increasing oil prices and extensive oil consumption accompanied by the potentially serious environmental impacts of oil extraction, the use of renewable biofuels as a partial replacement for traditional fossil fuels has gained recent worldwide attention. Among all of the alternatives, butanol obtained from the acetone-butanol- ethanol (ABE) process through a biological approach has become a potential replacement fuel (Yen *et al*., 2011; Bankar *et al*., 2011; Nakayama *et al*., 2011; Survase *et al*., 2011; Jones *et al.,* 2011; Yu *et al.,* 2011). The shortage of gasoline, even in some oil producing countries, has stimulated governments of countries like China to re-awaken their biofuel programme (Dong *et al*., 2011). Clostridial organisms such as *C. acetobutylicum, C. beijerinckii, C. saccharobutylicum*, and *C. saccharoperbutylacetonicum* are known for producing biofuels (Jones and Kreis, 1995). Genetic manipulation of these bacteria through gene knockout/ down systems have been developed through homologues recombination methods based on replicable/ non replicable vectors (Harris *et al*., 2002; Tomas *et al*., 2004; Heap *et al*., 2007; Papoutsakis, 2008; Heap and Minton, 2009; Kuehne *et al*., 2011; Lütke-Eversloh and Bahl, 2011). Antisense RNA strategies have also been employed to manipulate the genetic code of clostridia for biofuel production (Desai and Papoutsakis, 1999; Tummala *et al.,* 2003). *C. acetobutylicum* entire genome has been sequenced in an attempt to identify candidate genes for the purpose of genetic manipulation for biofuel production and other purposes (Bao *et al*., 2011). Biofuel tolerant mutants have been developed to enhance biofuel production by some non-pathogenic clostridia (Mao *et al.,* 2011). Apart from the usefulness of non-pathogenic clostridia in biofuel production, it is believed that their spores could serve as vehicles for systematic colonization of tumor cells in cancer therapy (Fox *et al*., 2000; Barbe´ *et al.,* 2006).

4. Clostridial Organisms as Agents of Bioterrorism

Clostridial organisms are targets for bioterrorism in our changing world where microorganisms have been used as agents of biological attack. Botulinum toxin in bioterrorism has been reviewed (Bossi *et al*., 2006). Aerosols of botulinum toxin could be used as a biological weapon (Franz *et al.,* 1997; Lecour *et al.,* 1998; Arnon *et al*., 2001). Deliberate release may also involve contamination of food or water supplies with toxin or *C. botulinum* bacteria. Botulinum toxin is extremely lethal and easy to produce. The Aum Shinrikyo cult in Japan attempted unsuccessfully to release an airborne form of botulinum toxin in Tokyo on three separate occasions in the early 1990s (Arnon *et al*., 2001). It is likely that several countries have developed and stockpiled botulinum toxin weapons (Arnon *et al.,* 2001). It has been estimated that a point-source aerosol release of botulinum toxin could incapacitate or kill 10% of the population. *C. botulinum* is a large, Gram-positive, strictly anaerobic bacillus that forms a subterminal spore. These spores can be found in soil samples and marine sediments throughout the world. Four groups of *C. botulinum* are described. Group I organisms are proteolytic in culture and produce toxin types A, B or F; group II organisms are nonproteolytic and produce toxin types B, E or F; group III produces toxin types C or D, and group IV toxins type G. These toxins are proteins of approximately 150 kD molecular weight and induce similar effects whether inhaled or ingested. When ingested, toxins are absorbed in the duodenum and jejunum, and enter into the bloodstream, and eventually reach peripheral cholinergic synapses. Botulinum toxin does not penetrate intact skin (Arnon *et al.,* 2001). Toxins act by binding to the presynaptic nerve terminal at the neuromuscular junction and at cholinergic autonomic sites. This binding prevents release of acetylcholine and interrupts neuro-transmission (Humeau *et al*., 2000). Human botulism is almost always caused by toxin types A, B, E and, in rare cases, F. Types C and D are associated with disease in birds and mammals. Type G is not associated with any disease in humans or animals. It has been estimated that, weight for weight, these toxins are the most toxic compounds known, with an estimated toxic dose, for toxin type A, of only 0.001 µg/kg of body weight when administered intravenously, subcutaneously or intraperitoneally (Middlebrook and Franz, 1997). By inhalation, the dose that would kill 50% of exposed persons (LD50) is 0.003 µg/kg of body weight. This toxin is 100,000 times more toxic than sarin gas (Fanz *et al*., 1997).

C. botulinum has been classified as a category A biological warfare agent (Johnson and Bradshaw, 2001). There are seven serologically distinct serotypes of the neurotoxin secreted by this bacterium (A–G), with types

A, B, E, and rarely F responsible for human intoxication. Botulinum neurotoxin (BoNT) A is the deadliest of the seven toxins with potency approximately one million times greater than cobra toxin, and one hundred billion times greater than cyanide. The LD50 for humans is approximately 1 ng/ kg of body weight (Schantz and Johnson, 1992). Eubanks *et al.* (2007) outlined the detailed technological advancement for the detection and protection against botulism as a biological warfare agent.

C. perfringens epsilon toxin, has been classified as category B agents (http://www.niaid. nih.gov/ biodefense/bandc_priority.htmj for classification of bio-threat agents) (Marks, 2004). Both *C. perfringens* spores and toxins have reportedly been considered as biological warfare agents (Mangold and Goldberg, 1999; Butler, 1999). Like *B. anthracis* spores, the spores of *C. perfringens* are robust and heat stable, but unlike *B. anthracis* spores there is no evidence that the spores can be delivered by the airborne route to cause disease. Rather, the spores have been considered as infective agents in weapons which cause traumatic injury. Flechette (shrapnel) weapons developed by Japan during World War II resulted in the delivery of spores into wounds and the subsequent establishment of gas gangrene (Mangold and Goldberg, 1999). The treatment of gas gangrene is notoriously difficult, because the infection becomes established in tissues that are deprived of blood. Evidence that the toxins produced by *C. perfringens* are potential biological warfare agents is still a course of dispute. However, it is believed that Iraq had a strong interest in *C. perfringens* toxins as biological weapons (Butler, 1999) and *C. perfringens* toxin is now placed on the list of CDC select agents as a toxin of particular concern. This toxin is active in a wide range of animal species and plays a central role in enterotoxaemia of sheep and lambs (Titball, 2009).

5. Epidemiology and Economic Importance of Clostridial Infections

Clostridium, a eubacteria, consists of diverse species whose economic importance includes the ability to cause disease (pathogenic strains), industrial uses and therapeutic potentials. The pathogenic strains which cause very lethal infections include *C. difficile, botulinum, perfringens, septicum, chauvoei*, etc. Clostridial infections cut across animal and human populations, with zoonotic species/ strains posing a great threat to community health in endemic areas. *C. difficile* infection is an increasing burden to the health care system, totaling more than $1 billion/ year in the United States of America alone (Leffler and Lamont, 2009).

Life-threatening soft tissue infections caused by *Clostridium* species have been described in the medical literature for hundreds of years largely because of their fulminant nature, distinctive clinical presentations and complex management issues. *C. perfringens*, *C. septicum* and *C. histolyticum* are the principal causes of trauma-associated gas gangrene and their incidence increases dramatically in times of war, hurricanes, earthquakes and other mass casualty conditions (Stevens *et al.*, 2011). Currently, tetanus occurs in about 1,000,000 people annually worldwide, and most patients are newly born in developing countries located in tropical areas. The World Health Organization (WHO) estimated that there were 715,000 deaths from tetanus among newborns in 1990. Neonatal tetanus is considered endemic in 90 developing countries and resulted in 248,000 deaths in 1997 (World Health Organization; http://www.who.int/vaccine-diseases/ NeonatallTetanus.shtml). Tetanus continues to cause ~250,000 deaths worldwide each year, predominantly in low- and middle income countries (Alam *et al*., 2008).

The life threatening consequences of clostridial organisms have been reviewed (Stevens *et al.*, 2011). During the civil war, 50% of soldiers who were wounded ultimately died. If the soldier was not killed outright, the extensive destruction of tissue inflicted by the 45 caliber lead bullets used at the time predisposed him to the development of gas gangrene, largely due to the resultant vascular damage. Civil war surgeons had few resources such as anaesthesia, intravenous fluids and antibiotics and thus “prophylactic amputation” became common place. During World War I, medical treatments had improved. However, weaponry also became more sophisticated. In this period, the incidence of gas gangrene was as high as 10% of wounded soldiers (MacLennan, 1962). Gas gangrene also claimed an estimated 100,000 German soldiers during this period.

In World War II, medical evacuation, improved surgical debridement techniques in field hospitals, gas gangrene anti-toxin and antibiotics were available and reduced the overall incidence of gas gangrene. For example, death from gas gangrene in US casualties in the western front of Europe was 8 cases/1000 wounded, for the Free French Forces it was 12.3/1000 and among prisoners of war, 51.9/1000 wounded. The much higher rate among prisoners was attributed to a delay in definitive surgical treatment which was 1 day for US casualties and 3.5 days for prisoners (MacLennan, 1962). In addition, the incidence of gas gangrene was higher in European theaters than in desert operations, likely because the fertile valleys of Europe were heavily contaminated with *C. perfringens* (50,000 per gram of soil), whereas they were rarely recovered in the Sahara Desert. Uniforms too were found to be contaminated with enteric clostridia and *C. perfringens* was isolated from 24% of shirts and 44% of trousers (McLennan, 1943a,b,c).

In the Korean, Vietnam, Gulf and Afghanistan wars, rapid evacuation, thorough (but not radical) surgical debridement, vascular reconstruction, improved supportive measures and greater antibiotic efficacy and availability greatly reduced the incidence of *C. perfringens* gas gangrene. Gas gangrene continues to be problematic in both military theaters and the civilian sectors, always associated with traumatic injuries. For instance, among nearly 2800 patients hospitalized following the 2008 Wenchuan earthquake in China, over 2.4% developed gas gangrene (Chen *et al.*, 2011). Interestingly, *C. septicum* as a cause of non-traumatic

gas gangrene has increased in civilian populations in recent years and necrotizing infections associated with skin-popping black tar heroin have been attributed to *C. perfringens, C. novyi* and *C. sordellii. C. sordellii* has been reported not only in trauma cases but also associated with pregnant females undergoing normal vaginal delivery, caesarian sections or medical abortions. *C. difficile*, the causative agent of antimicrobial- associated diarrhoea and pseudo-membranous colitis, is currently one of the most important nosocomial pathogens because of its zoonotic nature (Rupnik *et al*., 2009). Nosocomial infections with the Gram-positive pathogen *C. difficile* pose a major risk for hospitalized patients and result in significant costs to health care systems.

Blackleg is known to be an endemic disease in both developed and developing countries of the world and is a well-known cause of financial loss to cattle raisers in many parts of the world (Adams, 1998). The economic losses of ruminants to the disease have yet to be quantified in most countries, but in Nigeria, losses of Zebu cattle to the disease have been estimated at US$4.3 million annually (Useh *et al*., 2006b). In United States of America, Latin America, India and other parts of Asia and Europe, the economic losses of ruminants to blackleg have yet to be estimated, but it has been reported that the disease causes major economic losses in cattle and minor losses in sheep (Cottral, 1978; Ramarao and Rao, 1990; Troxel *et al*., 1997; Flyod, 1994; Adams, 1998).

6. Management, Prevention and Control of Infections by Pathogenic Clostridia

Resistance of pathogenic clostridial organisms to therapeutic antimicrobials has been reported and is increasingly becoming an issue of concern to public health (Gamboa-Coronado *et al.,* 2011). The resistance means that alternative therapeutic protocols will have to be developed in the future to treat resistant strains. Management of *C. difficile* infection in human beings has been reviewed (Leffler and Lamont, 2009). Drugs like metronidazole, (oral administration), vancomycin and several stand-alone therapies or as adjunct agents have been used. Alternative treatments include the use of rifaximin (Johnson *et al.,* 2007; Garey *et al.,* 2008). Immunotherapy (Kyne *et al*., 2001), microbiologic therapy (Seal *et al.,* 1987) and probiotic therapy (McFarland, 2006) have also been carried out. Prevention and control is better achieved by appropriate use of antibiotics, especially broad spectrum agents and those shown to have a particular predisposition for *C. difficile* infection. Treatment of clostridial infections caused by the highly toxigenic strains involves neutralization of the toxins.

The drug of choice for treatment of blackleg is procaine penicillin (10 000 IU/kg) over a 3–5 day therapy period. It is generally advocated that the administration of crystalline penicillins intravenously should precede the long-acting preparations that should be administered directly in the affected tissues (Radostits *et al*., 2000). The best preventive and control strategy is vaccination.

7. Natural Products as Potential Therapeutic Agents Against Clostridial Infections

Globally, millions of people in the developing world rely on medicinal plants for primary health care, income generation and livelihood improvement (WHO, 2002). The active substances present in many medicinal plants could be used as therapeutic alternatives against clostridial infections (Woodford and Livermore, 2009). Although the mechanisms of pharmacological actions of most medicinal plants with potentials to ameliorate clostridial infections have yet to be defined, there are strong indications that the results of most *in vitro* studies that seem to point to some beneficial actions of these plants is worth further investigations after all. Table 1 shows a list of medicinal plants from different parts of the world with potential therapeutic properties against clostridial infections.

C. perfringens is classified into five serotypes (A, B, C, D and E) based on the type of enterotoxins it produces. Delta-toxin is one of the five haemolysins released by *Clostridium perfringens* which play a most important role in gas gangrene and gastroenteritis. The native structure of the delta-toxin is not yet reported in the structural databases (Skariyachan *et al*., 2011). *C. perfringens* has been shown to develop multiple drug resistance, indicating that the treatment for this bacterium is quite challenging. There is need, therefore, to employ the use of alternative therapeutic agents. The rational design of improved therapeutics requires the crystal structure for the toxin. However, the structure for the toxin is not yet available in its native form. In a recent study, some authors modeled the toxin structure using α- haemolysin of *Staphylococcus aureus* (PDB: 3M4D chain A) as template. The docking of the toxin with the herbal extract curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione) showed a binding energy of -8.6 Kcal/mol, in comparison to the known antibiotic linezolid with binding energy of -6.1 Kcal/mol. This data is believed to be relevant in the future design and development of novel compounds against the delta-toxin produced by *C. perfringens*.

Table 1. List of some natural products with ethnopharmacological potentials against clostridial infections

Natural product	possible clostridial target	Reference
Combretum fragrans	*C. chauvoei*	Useh *et al*. (2004)
Aristolochia paucinervis	*C. perfringens/ difficile*	Gadhi *et al*. (1999)
Melia toosendan	*C. botulinum*	Shi & Li (2007);Nakai *et al*. (2009)
Propolis (bee glue)	*Clostridium perfringens*	Boyanova *et al*. (2006)
Clostridium difficile		"
Clostridium tertium		"
Other *Clostridium* species		"
Phyllanthus muellerianus	*C. sporogenes*	Brusotti *et al*. (2011)
Tamarindus indicus	*C. chauvoei*	Useh *et al*. (2004)
Galla Rhois	*Clostridium perfringens, C. paraputrificum*	Ahn *et al*. (1998)
Glycyrrhiza glabra linn	*Clostridium* spp	Saxena (2005)
Ghee & cocoanut	*C. tetanii*	Hlady *et al*. (1992; Bennett *et al*. (1997)
Teas & tea flavonoids	*C. perfringens*	Friedman, 2007
Korean dung beetle	*C. difficile*	Kang *et al*. (2011)
Clematis hirsute	*C. chauvoei*	*Yineger et al.* (2007)
Rumex nepalensis	*C. chauvoei*	"
Leonotis ocymifolia	*Anthrax bacillus*	"
Cucumis ficifolius	*C. chauvoei*	"
Nigella sativa L.	*C.chauvoei*	"
Discopodium eremanthum Chiov.	*C. chauvoei*	"
Sonchus bipontini	*C. chauvoei*	"
Crepis rueppellii	*C. chauvoei*	"
Nicotiana tabaccum L.	*C. chauvoei*	"
Ruta chalepensis L	"	"
Sida schimperiana.	"	"
Sonchus bipontini	"	"
Cymbopogon citrates	"	"
Alchemilla abyssinica	"	"
Salvia nilotica	"	"
Vernonia myrantha	"	"
Senecio fresenii	"	"
Curcumin (from *Curcuma longa*) docked with α-toxin of *S. aureus*	*C. perfringens* δ- toxin	Skariyachan *et al*. (2011)
Tea phenolics	*C. difficile*	Lee *et al*. (2006)
Thearubigin	*C. botulinum*	Satoh *et al*. (2001)

It has been reported that tea catechins strongly inhibit the growth of *C. perfringens in vitro* (Hara *et al*., 1989; Ahn *et al*., 1991; Hara *et al*., 1995). Green tea catechins also reduced the heat-resistance characteristics of *C. hermoaceticum*, which proliferate in vending machines, causing sour spoilage in milk and other drinks (Sakanaka *et al*., 2000). Some other unpublished data (cited by Friedman, 2007) also suggest that concentrated green tea extracts inhibit sporulation and growth of *C. perfringens* in ground meat and turkey products during chilling. These observations suggest that the use of polyphenols or other natural antimicrobials may result in reduction of temperatures used in thermal processing of foods.

Coprisin, an antibacterial peptide has been isolated from *Copris tripartitus*, a Korean dung beetle, and a nine-amino-acid peptide identified in its α-helical region (Hwang *et al*., 2009). Kang *et al.* (2011) investigated the clinical significance of treatment with a coprisin analogue (a disulfide dimer of the nine peptides) on inflammation and mucosal damage in a mouse model of acute gut inflammation, established by administration of antibiotics followed by *C. difficile* infection. Coprisin treatment

significantly ameliorated decreases in body weight, improved survival rate, decreased mucosal damage and pro-inflammatory cytokine production. In contrast, the coprisin analogue had no apparent antibiotic activity against commensal bacteria, including *Lactobacillus* and *Bifidobacterium* spp, which are known to inhibit the colonization of the gut by *C. difficile*. The exposure of *C. difficile* to the coprisin analogue caused a marked increase in nuclear propidium iodide (PI) staining, indicating membrane damage. The staining levels were similar to those seen with bacteria treated with a positive control for membrane disruption (EDTA). In contrast, coprisin analogue treatment did not trigger increases in the nuclear PI staining of *Bifidobacterium thermophilum*. This observation suggests that the antibiotic activity of the coprisin analogue may occur through specific membrane disruption of *C. difficile*. Thus, these results indicate that the coprisin analogue may prove useful as a therapeutic agent for *C. difficile* -associated inflammatory diarrhoea and pseudo-membranous colitis.

Chinese traditional medicine has a long history which dates back thousands of years ago. To date, Chinese people still believe in traditional medicine and herbal medications are very popular in Chinese societies around the world. The latest encyclopedia of Chinese traditional medicine lists over 5500 natural sources (82.8% of which are plants) that form the basis for the 100,000–500,000 prescriptions of Chinese traditional medicine (Tang and Eisenbrand, 1992; Zhu and Woerdenbag, 1995). However, Chinese traditional medicine is unacceptable to Western society partly because the bases of Chinese traditional medicine like the yin-yang theory and five element theory (Cheng, 2000) are difficult to understand. Chinese herbal medicine has shown that the fruit and bark of plants belonging to the family Melia could be used as digestive tract-parasiticide and agricultural insecticide. Toosendanin (TSN, C30H38O11, FW=574), a triterpenoid derivative, was extracted from the bark of *Melia toosendan* Sieb. et Zucc. by Chinese scientists in 1950's and used as an ascarifuge in China instead of imported sendanin. Studies have demonstrated that TSN possesses special biological actions and perhaps considerable pharmacological values in scientific research, clinical medicine and agriculture. The first is that by interfering with neurotransmitter release by causing an initial facilitation, TSN eventually blocks synaptic transmission at both the neuromuscular junction and central synapses. The action might result from TSN-induced Ca^{2+}-sensitivity change and final elimination of transmitter release machinery. The second is that despite sharing many similar actions with botulinum neurotoxin (BoNT) on blocking neuromuscular transmission, TSN has a markedly anti-botulismic action *in vivo* and *in vitro*: TSN-treatment is reported to prevent death in mice and monkeys with botulism. TSN-incubation *in vitro* or TSN-injection *in vivo* endows neuromuscular junction with a high tolerance to BoNT. Studies suggest that the anti-botulismic action is achieved by preventing BoNT from approaching its enzymatic substrate, SNARE protein (Shi and Li, 2007).

Other studies on the role of TSN as anti-botulism natural product have been reported (Nakai *et al.*, 2009). Experiments carried out on phrenic nerve-diaphragm preparations of rats showed that the neuromuscular blocking action of TSN was similar to that of botulinum neurotoxin (BoNT) in many respects (Shi *et al*., 1980, 1981a,b, 1982). For example, they were both concentration- and temperature dependent; and the temperature coefficients were both high. Also, their dosage-response relationships were similar and the blocking effects were both not only irreversible but also related to the nerve activity and concentration of Ca^{2+}. There was always a delay between drug application and transmission block. Even if the drugs were washed out during the latent period, the blockade still occurred, indicating that both TSN and BoNT bound to their binding sites very fast. Similar to BoNT, TSN eventually blocked neurotransmitter release by abolishing Ca^{2+}-sensitivity of the transmitter release machinery. Moreover, some drugs, like guanidine and 4-aminopyridine, which facilitates neurotransmitter release and antagonize botulism, also have anti-TSN effect (Shi *et al*., 1981a). Except for sharing these similarities, the action of TSN is different from that of BoNT in some respects. For example, TSN changed excitability and fine structure of nerve endings and affected neurotransmitter release at central synapses as it did at the neuromuscular junction (Huang *et al.*, 1996; Shi and Chen, 1999; Chen *et al.*, 1999; Xu *et al*., 2004). The most prominent difference was that TSN always facilitated neurotransmitter release before blocking it. During the facilitatory phase, both the transmitter release (either spontaneous or evoked), and the Ca^{2+}- sensitivity of transmitter release machinery were enhanced (Shi *et al.*, 1981a, 1982; Xu *et al*., 2004). The similarities between the actions of TSN and BoNT suggest that TSN and BoNT may act at an adjacent site by a similar approach. However, analysis of their differences may give us a clue with which to reveal the anti-botulismic mechanism of TSN.

C. chauvoei, which is a very important veterinary pathogen is known to produce sialidase (neuraminidase), an enzyme reported to facilitate its spread in host tissues (Useh *et al*., 2004a; Useh *et al.*, 2006c). Many herbal remedies have been reported to ameliorate blackleg, caused by *C. chauvoei*, but the rational basis for this has yet to be demonstrated in the literature (Yineger *et al*., 2007). The Fulani pastoralists of rural Nigeria, who own livestock resources in the country, prefer the use of herbal remedies to treat animal diseases, including blackleg, because they are more natural, cheaper and safer (Jagun *et al.*, 1996; Gammaniel, 2000). *Tamarindus indicus* and *Combretum fragrans* are two common herbal remedies used recurrently by the nomads of rural Nigeria to treat blackleg (Abdu *et al.*, 2000). A study by some Nigerian authors to investigate the possible role of the herbs on blackleg showed that methanolic extracts of the stem barks of these plants inhibited sialidase (neuraminidase) activity *in vitro* in a dose-dependent manner. The authors therefore suspected that neuraminidase inhibition, leading to inability of the bacteria to spread in tissues, could be a major mechanism by which the herbs ameliorated the disease. The results revealed a target of action by the medicinal plants, thus corroborating their use in traditional medicine practices in Nigeria (Useh *et al*.,

2004b). Sialidases (neuraminidases) have been implicated in the pathologies of several diseases, including cell invasion in Chaga's disease, anaemia in trypanosomosis and viral invasion in Newcastle disease. The foregoing, according to the authors, makes the sourcing of neuraminidase inhibitors mandatory for treatment and amelioration of clinical symptoms related to the physiological activity of the enzyme. In most cases, the inhibitors are synthetic and indeed costly, e.g. Zanamivir used in the treatment of influenza virus infection. Moreover, some of the synthetic inhibitors become less effective on account of mutations, which are rampant in sialidases. This makes recourse to plants as source of neuraminidase inhibitors an appealing alternative, because their active compounds are synthesized in direct response to bacterial and viral invasion. In the said study, the two medicinal plants were tested, because of their reported role in ameliorating blackleg in traditional veterinary practice. These authors concluded that the active principles of these medicinal plants should be further characterized, to exploit the findings in drug development.

8. Conclusion

The future use of natural products in the clinical management of clostridial infections, although laced with a lot of potentials, is a puzzle that research should be intensified to resolve, considering the economic importance, with regards to the pathogenic, industrial and potential therapeutic uses of members of this genus. The emergence of drug resistant strains, with enhanced pathogenicity is deadly and requires concerted efforts to pull through this quagmire. Medical and veterinary research should be re-jigged to evolve active principles that are not so easily rendered inconsequential by the surge in drug resistant strains. Future research should focus on the possibility of targeting the critical virulence factors of clostridial agents using active principles from herbs, which have proved to be useful and potential therapeutic agents over time.

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