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Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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African Journal of Microbiology Research

Review

Review on common foodborne pathogens in Ethiopia

Edget Abayneh[1], Dagmar Nölkes[1] and Biruhtesfa Asrade[2]*

[1]College of Veterinary Medicine, Haramaya University, Ethiopia.
[2]School of Veterinary Medicine, Hawassa University, Ethiopia.

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Foodborne pathogens are among the common causes of illness and death as well as public health problem which result in the loss of labor force both in developed and developing countries. The World Health Organization estimated that in developed countries, up to 30% of the population suffers from foodborne diseases each year, whereas in developing countries up to 70% of cases of diarrheal disease are associated with the consumption of contaminated food per year. Animal products such as meats, fish and their products are generally regarded as high-risk commodity in respect of pathogen contents, natural toxins and other possible contaminants. In Ethiopia, the widespread habit of raw beef consumption is a potential cause for foodborne illnesses besides, the common factors such as overcrowding, poverty, inadequate sanitary conditions, and poor general hygiene. In Ethiopia, as in other developing countries, it is difficult to evaluate the burden of food borne pathogens because of the limited scope of studies and lack of coordinated epidemiological surveillance systems. In addition, under-reporting of cases and the presence of other diseases considered to be of high priority may have overshadowed the problem of foodborne pathogens. This review focused on published report of common food borne pathogen specifically *Salmonella* spp., *Escherichia coli*, *Listeria* spp., *Staphylococcus* spp. and *Campylobacter* spp. in different parts of Ethiopia.

Key words: *Campylobacter* spp., *Escherichia coli*, Ethiopia, foodborne pathogen, *Listeria* spp., *Salmonella* spp., *Staphylococcus* spp.

INTRODUCTION

Foodborne pathogens are one of the leading causes of illness and death in developing countries resulting in the loss of labor force which could have contributed in the economic growth (Fratamico et al., 2005).

Food borne diseases occur particularly in Africa because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment and lack of education for food-handlers (WHO, 2004). Of the foods intended for humans, those of animal origin tend to be most hazardous unless the principles of food hygiene are employed. Animal products such as meats, fish and their products are generally regarded as high-risk commodity in respect of pathogen contents, natural toxins and other possible contaminants is an unavoidable consequence of meat processing (Jones et al., 2008). Data regarding meat borne diseases in Ethiopia are not well documented among which studies conducted

*Corresponding author. E-mail: biruhta@gmail.com.

in different parts of the country have shown the public health importance of several bacterial pathogens associated with foods of animal origin (Bayleyegn et al., 2003; Ejeta et al., 2004; Adem et al., 2008; Kumar et al., 2009; Tefera et al., 2009).

In Ethiopia, like other developing countries, it is difficult to evaluate the burden of food borne pathogens because of the limited scope of studies and lack of coordinated epidemiological surveillance systems. In addition, under-reporting of cases and the presence of other diseases considered to be of high priority may have overshadowed the problem of foodborne pathogens (Oosterom, 1991).

The widespread habit of raw beef consumption is a potential cause for food borne illnesses in Ethiopia, besides the common factors such as overcrowding, poverty, inadequate sanitary conditions and poor general hygiene (Siddiqui et al., 2006).

In Ethiopia, there have been several studies conducted on foodborne pathogens among which are *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, Staphylococcus *aureus* and *Campylobacter* spp. but there is no compiled document for easy access. Therefore, the objectives of this review paper are: To provide well organized data on available research works (published) on common foodborne pathogens in Ethiopia. And to show research gaps on foodborne pathogens in Ethiopia.

Salmonella spp.

Salmonella are the major food borne pathogenic bacteria in humans as well as in animals. *Salmonella* species are leading causes of acute gastroenteritis in several countries and salmonellosis remains an important public health problem worldwide, particularly in the developing countries (Rotimi et al., 2008). Salmonellosis is the most common food borne disease in both developing and developed countries, although incidence rates vary according to the country (Stevens et al., 2006). The fecal wastes from infected animals and humans are important sources of bacterial contamination of the environment and the food chain (Ponce et al., 2008).

Salmonella infection in meat animals, including cattle, swine and sheep, arises from intensive rearing practices and the use of contaminated feeds (D'Aoust, 1989). Cross-contamination of carcasses with *Salmonella* can also occur during slaughtering operations. Stress associated with transport of animals to abattoir augments shedding of *Salmonella* by carrier animals and this may contribute to the spread of the organism to other animals in the slaughter plant (Baird-Parker, 1990; Isaacson et al., 1999). Slaughtering procedures potentially involve many risks of both direct and cross-contamination of carcasses and meat surfaces. During slaughter, faecal contamination of edible organs with subsequent contamination of the carcass may occur. This can be carried through all slaughter procedures up to the processing of the raw products, which are important sources of *Salmonella* in the human food chain (Edwards et al., 1997).

It is usually difficult to evaluate the situation of salmonellosis in developing countries because of the very limited scope of studies and lack of coordinated epidemiological surveillance systems (Oosterom, 1991; Ache and Szyfres, 2001). In addition, under reporting of cases and presence of other diseases considered to be of high priority may have overshadowed the problem of salmonellosis in some developing countries including Ethiopia. The increased global population coupled with mass production of animal and animal food and the rapid international trade in agriculture, aquaculture and food products could worsen the problem (D'Aoust, 1994).

A periodic surveillance of the level of *Salmonella* contamination in the different food animals, food products and environment is necessary to control the spread of the pathogen and infection of man (Dawson, 1992). Therefore, the different studies conducted on food borne salmonellosis in different parts of Ethiopia by different researcher are systematically summarized and presented in Table 1.

From 2000-2013 almost 15 different studies were published on foodborne Salmonellosis which are concentrated in some parts of Ethiopia especially in Addis Ababa and Debre Zeit with 8 studies in Addis Ababa, 6 in Debre Zeit. There might be unpublished studies done in other place which helps to provide holistic figure of the overall foodborne Salmonellosis patterns in Ethiopia. As a recommendation, it is better to do region wide research to provide a representative estimate of foodborne Salmonellosis in Ethiopia.

Escherichia coli

Infection with *E. coli*O157:H7 is a major food borne and zoonotic pathogen responsible for hemorrhagic colitis and hemolytic uremic syndromes in humans. Transmission to human occurs through consumption of undercooked meat, unpasteurized dairy products, and vegetables or water contaminated by feces of carrier animals (Songer and Post, 2005).

Meats are a common source of *E. coli* contamination, which may be acquired during slaughter through fecal contact. *E. coli* outbreaks have been associated with meat (especially ground beef), dairy products, mayonnaise, apple cider, sprouts (radish), lettuce and spinach. *E. coli* outbreaks have also been associated with swimming pools and nursing schools (Arun, 2008).

Verocytotoxigenic *E. coli* (VTEC) (also referred to as Shiga toxin-producing *E. coli*), including serotype O157:H7, are one of such group, causing severe, chronic, and potentially fatal illness such as hemorrhagic colitis, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura and, in severe cases, death, related

Table 1. Systematic summary of publications on foodborne Salmonellosis in Ethiopia.

Location	Sample/source	Prevalence (%)	Serotypes	Antimicrobial susceptibility profile	References
D/Zeit	Feces MLN Abdo.muscle Diaph.muscle	2/323(0.6) 4/323(1.2) 9/323(2.8) 10/323(3.1)	*S.* Mishmarhaemek *S.* Typhimurium *S.* Enteritidis *S.* Guildford *S.* Dublin	Resistant to AMP, CEP, SMX, TIC, TET	Alemayehu et al. (2003)
A/A D/Zeit Dire–dawa Jigijiga	**Slaughtered cattle** feces MLNs Muscle **Slaughtered Camel** Feces MLNs Liver Spleen Muscle **Slaughter house personnel** Human stool **Supermarket** Minced beef **Chicken meat and giblet** Meat Liver Gizzard Heart	 7/370(1.9) 9/370(2.4) 63/1116(5.6) 18/119(15.1) 19/119(15.9) 14/119(11.8) 17/119(14.3) 48/238(16.2) 18/300(6) 46/380(12.1) 54/452(8.3) 33/111(29.7) 48/116(41.4) 18/85(21.2)	*S.* Braenderup *S.* Dublin *S.* Saintpaul *S.* Typhimurium *S.* Typhimurium var. Copenhagen *S.* Anatum		Bayleyegn et al. (2003)
A/A D/Zeit	Chicken meat Skin Liver Gizzard Heart	16/104(15.4) 8/104(7.7) 19/55(34.5) 23/56(41.1) 14/59(23.7)	*S.* Braendrup, Typhimuriumvar Copenhagen, Anatum, Kottbus, Typhimurium	Resistance to CEX	Molla and Mesfin (2003)

Table 1. Contd.

A/A	Minced beef	23/160(14.4)	*S.* Infantis, Braenderup, dublin, Saintpaul, Bovismorbificans, Anatum, vojle, *S.*I:8, 20		Ejeta et al. (2004)
	Mutton	12/85(14.1)	*S.* Infantis, Braenderup, Anatum, Bovismorbificans & *S.* I:47, z4, z23		
	Pork	9/55(16.4)	*S.* Infantis, Braenderup & Vejle		
D/Zeit	Feces	5/107(4.7)	*S.* Infantis, Butantan		Woldemariam et al. (2005)
	MLN	3/107(2.8)	*S.* Infantis, Anatum, Zanzibar, Butantan, Typhimurium & Kingabowa		
	Liver	2/107(1.9)	*S.* Infantis, Butantan, Braenderup & Kottbus		
	Spleen	7/107(6.5)	*S.* Infantis & Butantan		
	Diagh.muscle	9/107(8.4)	*S.* Infantis & Butantan & Gingabwa		
	Abdo.muscle	7/107(6.5)	*S.* Infantis, Braenderup & Butantan		
D/Zeit	Pork	94/501(18.8)	*S.* Anatum, Newport, Enteritidis, Hadar, Uganda, Eastnourne & Kentucky	Multidrug resistant (*S.hadar* highest).	Molla et al. (2006a)
A/A Modjo	Feces	7/204(3.4)	*S.* Typhimurium, Give, Herdelberg, Reading, Poona & Enteritidis	*S.typhimurium* (STR, sulfisoxazole, TET, TMP) *S. reading* (STR, SUL, TET).	Molla et al. (2006b)
	MLN	10/204(4.9)			
	Liver	2/204(0.9)			
	Spleen	1/204(0.5)			
	Abdo. Muscle	2/204(0.9)			
	Diagh. Muscle	0/204			

Table 1. Contd.

A/A	cecal content MLN Carcass swab	63/278(23) 99/278(36) 11/278(4)	*S.* Hadar, Eastnourne, Saintpaul, Typhimurium, & Var. Copenhagen, Enteritidis, Newport & Anatum.	Substantially multidrug resistant	Aragaw et al. (2007)
A/A	Chicken meat	29/208(13.9)	*S.* Braenderup, Hadar, Newport, Kentucky, Typhimurium , Bovismorbificans & Anatum.	*S. braenderup*(AMP, SPT, STR, SUL, SXT, TMP)	Zewdu and Cornelius (2009)
	Pork	22/194(11.3)	*S.* Newport, Haifa, Dublin , Infantis & Kottbus.	*S. kentucky* (AMP, AMC, CEF, CIP, GEN, NAL, SPT, STR, SUL, TTC)	
	Minced beef	12/142(8.5)	*S.* Newport, Dublin, Anatum, Infantis, Typhimurium, Kentucky & Saintpaul		
	Mutton	23/212(10.8)	*S.* Newport, Hadar, Typhimurium, Dublin, Bovismorbificans, Infantis & Zanzibar	*S. Dublin* (CRB, TTC)	
	Cottage cheese	4/190(2.1)	S.Newport & Haifa		
	Fish	3/128(2.3)	*S.* Newport & Zanzibar		
	Ice cream	0/126			
	Stool sample	5/68(7.4)	*S.* Newport		
A/A	Lettuce	8/40(20)	*Salmonella* spp.	All *salmonella* isolates resistant (PEN and AMC)	Biniam and Mogessie (2010)
	Green paper	4/40(10)			

Table 1. Contd.

D/Zeit	Hide swab	31/100(31)	*S.* Anatum	(STR, TTC)	Sibhat et al. (2011)
	Hand swab(at fly	7/100(7)	*S.* Newport	(TTC, STR, SUL)	
	Hand swab (at evisceration)	2/100(2)	*S.* Eastbourne	(TTC)	
	Carcass	2/100(2)	*S.* Uganda	-	
B/Dar	Liver	2/186(1.1)	*S.* Typhimurium *S.* Infantis	Multidrug resistant	Alemu and Zewde (2012)
	MLN	6/186(3.2)	*S.* Newport		
	Carcass swab	9/186(4.8)	*S.* Hidelberg		
	Intestinal content sample	11/186(5.9)	*S.* Mishmarhaemak *S.* Haifa		
Mekelle	Margarine	0/10	*Salmonella* spp.		Mekonnen et al. (2012a)
	Mayonnaise	2/10(20)			
	Sardine	2/10(20)			
	“wot”	13/30(43.3)			
	Macaroni	2/10(20)			
	“fata”	0/30			
	“zahla”	4/10(40)			
	Mango juice	7/15(45)			
	Avocado juice	4/17(23.5)			
	Fruit mix	2/8(25)			
	Table scraping	11/110(10)			
B/Dar	Ready to eat white lupine	23/40(57.5)	*Salmonella* spp.	Highest resistance to ERY (86.9%)	Mulgeta and Million (2013)
A/A	Whole egg	18/384(4.6	*S.* Enteritidis	-	Zinabu et al. (2013)

A/A: Addis Ababa B/Dar: Bahir Dar D/Zeit: Debre Zeit (current name Bishoftu).

to their ability to produce one or more toxins known as verotoxin or Shiga toxin (Tarr, 1995). Consumption of raw or undercooked foods of bovine origin has been the most common means of transmitting VTEC organisms in sporadic cases and in outbreaks of VTEC infection (Uhitil et al., 2005).

Outbreaks of *E. coli*O157 have been reported in different parts of the world and antibiotic use is controversial because of the potential to increase production and secretion of Shiga toxins. Increase in antibiotic resistance has been noted over the last 20 years (Adem et al., 2008). Differentiation of pathogenic strains from the normal flora depends on the identification of virulence characteristics (OIE, 2008).

In Ethiopia, there were studies conducted by few researchers (Adem et al., 2008; Mersha et al., 2009; Taye et al., 2013) to determine the occurrence and proportion of *E. coli* O157:H7 in faeces, skin swabs and carcasses of sheep, goat and cattle in Debre Zeit, Modjo and Haramaya University. Even though little is known about the prevalence and antimicrobial susceptibility pattern of this bacterium in Ethiopia either in humans or animal population or foods, there is no information in eastern Ethiopia generally and in Haramaya

University and its surrounding specifically, where large populations of cattle are reared for slaughter Taye et al., 2013).

Studies done on foodborne *E. coli* infection are few in number and little is known about the public health effect of foodborne *E. coli* due to lack of well documentation system and integrated surveillance system in Ethiopia. This review will provide a systematic summary of those studies conducted by few researchers on foodborne *E. coli* infection (Table 2).

Different researches were conducted on foodborne *E. coli* based on abattoir sample, butcher shop, dairy milk and different food which are ready to eat from 2008-2014 only in limited parts of Ethiopia. Out of seventeen research, six specially emphasized on *E. coli* O157:H7 serogroups.

As a remark since *E. coli* O157:H7 is an emerging foodborne and zoonotic pathogen, researchers should emphasis on the public health significance of this pathogen to assess the overall prevalence and public health importance of food borne *E. coli* O157:H7 in Ethiopia.

Listeria monocytogenes

Listeriosis is one of the important emerging bacterial zoonotic diseases that occur in a variety of animals and humans. It arises mainly from the consumption of contaminated food products (Acha and Szyfres, 2001; Malik et al., 2002). Reports indicate that listeriosis has emerged to be more important in developed countries but is reported less frequently in developing countries (Todar, 2003). This could be associated with lack of awareness of laboratory technicians or lack of diagnostic facilities and limited resources together with the presence of other disease epidemics that claim more priority than listeriosis in developing countries including Ethiopia.

A number of food borne outbreaks caused by *L. monocytogenes*, have so far been reported, which were known to cause serial deaths in a number of individuals and in different regions, especially in Europe and the USA (Todar, 2003). However, in most African countries, there are a few reports on *Listeria* and listeriosis, as compared to the Europe and USA (Molla et al., 2004). This is because; the organism has not been given much attention, and may be due to lack of adequate facility, life style differences and RTE foods are more common in USA and Europe than in Africa regardless of the habit of consumption of raw milk and milk product as well as raw meat. Published information on the status of food borne listeriosis is very limited both in the veterinary and public health sectors in Ethiopia and those studies which are published are presented in well summarized manner (Table 3).

Few researches were done on foodborne *L. monocytogenes* and other *Listeria* spp. in Ethiopia from 2000- 2014 and all studies were done in Addis Ababa.

There may be unpublished study output which is kept on shelf only, so this will not represent the overall status of foodborne *L. monocytogenes* in Ethiopia. This is a wide research area for researchers with emphases given on public health significance, prevalence and antimicrobial susceptibility profile of *L. monocytogenes* since little is known about the burden of *L. monocytogenes* in food specifically in raw milk and meat product and habit of consuming raw milk and meat in Ethiopia.

Staphylococcus aureus

S. aureus is one of the most common causes of food borne intoxication in most countries of the world. *S. aureus* is a facultative anaerobic Gram-positive coccus, nonmotile, catalase and coagulase positive of the micrococcaceae family (Bhatia and Zahoor, 2007).

Convenience food offers a suitable growth environment for toxin-producing bacteria such as *S. aureus*, which is able to grow and express virulence in a wide variety of foods such as milk products, mixed foods, meat and meat products, egg and egg products, cakes and ice cream (Silva et al., 2001).

Various fatal diseases caused by street food intoxications have been lately reported (Sina et al., 2011). In reported street food epidemiology studies, *S. aureus* is the most predominant virulent bacteria responsible for a wide range of human diseases. It represents the major causal agent of food intoxication through its enterotoxin products (Le Loir et al., 2003). Several studies have been conducted in Ethiopia but there is no properly documented file so this review provides a published research output in summarized manner in Table 4.

Various researches has been done on foodborne *S. aureus* intoxication in certain parts of Ethiopia but little is known about the status of *S. aureus* due to less priority given by researchers and public health professionals both in human and veterinary medicine in Ethiopia at large. From 2000-2014 only nineteen published studies were done among which are 2 in Jimma, 2 in D/Zeit, 1 in Jigjiga, 3 in Mekelle, 2 in B/dar, 1 in Adami-Tulu, 1 in Adama, 1 in Shashemene, 1 in Gondar, 1 in Hawassa, 1 in Yabello, 1 in Humera and Abergelle and 2 in A/A. Since *S. aureus* is a highly zoonotic foodborne pathogen, due emphases should be given to assess and determine the overall prevalence, public health significant and antimicrobial susceptibility profile of foodborne *S. aureus* in Ethiopia.

Campylobacter spp.

Campylobacteriosis is historically a zoonotic disease found among cats, goats, poultry, calves, lambs and dogs. Although uncommon, human-to-human spread is also possible through faecal-oral route. The cross-contamination of foods during preparation is also likely to

Table 2. Systematic summary of publications on foodborne *E. coli* including *E. coli* O157:H7 in Ethiopia.

Location	Sample/source	Prevalence (%)	*E. coli* Serotype	Antimicrobial susceptibility profile	Reference
D/Zeit Modjo	Meat Beef Mutton Goat meat	20/250(8) 6/243(2.5) 5/245(2)	*E. coli* O157:H7	(KAN, STR, AMP, CEP, TTC, TRIM)	Hiko et al. (2008)
Modjo	Feces Skin swab Carcass before wash Carcass after wash Water	8/172(4.7) 15/172(8.7) 14/172(8.1) 15/172(8.7) 1/23(4.3)	*E. coli* O157:H7	-	Mersha et al. (2009)
Jimma	'Kitifo' Surface swab Carcass swab	120 12 33 44/165(26.6)	Thermo tolerant *E. coli* (84%)	-	Haimanot et al. (2010)
Jimma	Cow milk	164/218(75.22)	*E. coli* (9.17%) isolates	-	Tariku et al. (2011)
Gondar	Cow milk	164/322(50.9)	*E. coli* (4.3%)	ERY, AMP, TTC	Nibret et al. (2011)
B/dar	Cow milk	99/139(71.2)	*E. coli* (2.5%)	-	Molalign et al. (2011)
Shashemene	Cow milk	217/364(59.6)	*E. coli* (10.6%)	-	Desie et al. (2011)
Yabello	Cow milk	81/712(11.37)	*E. coli*	-	Adane et al. (2012)
Humera&Abergelle	Sheep milk Goat milk	135 255 84/390(21.5)	*E. coli* (17%)	-	G/Wahid et al. (2012)
Mekele	Margarine mayonnaise sardine "wot" Macaroni "fata" "Zahla" Mango juice Avocado juice Fruit mix Table scraping	4/10(40) 2/10(20) 0/10 4/30(13.4) 0/10 10/30(33.4) 2/10(20) 3/15(20) 7/17(41.1) 1/8(12.5) 32/110(29)	*E. coli*	-	Mekonnenet al. (2012a)

Table 2. Contd.

Mekele	Meat Butcher shop Abattoir Street meat sales	 2/30(6.7) 2/5(40) 3/5(60)	 *E. coli* *E. coli*32 (91.4%) *E. coli* O15:H7 3 (2.6%)	AMP, ERY, CL, NA, CHL, TRIM-SUL	Mekonnen et al. (2012b)
Mekele	Cow milk	128/174(73.56)	*E. coli* (27.3%)	*E.coli* (TTC (48.57%), C (28.86%), KEN (8.86%), SPT (5.7%), AMP (65.7%) AMC (66.67%))	Haftu et al. (2012)
Haramaya university	Carcass swab	35/113(30.97)	*E. coli*	*E.coli* O157:H7 (TTC (33.33%), AMP (100%), AMC (100%).	Taye et al. (2013)
B/dar	Ready to eat white lupin	29/40(72.5)	*E. coli*	Resistant to TTC	Mulugeta and Million (2013)
Holeta	Cow milk	183/224(81.7)	*E. coli* (11.6%)		Ayano et al. (2013)
A/A	Cow milk	80/118(67.8)	*E. coli* O157:H7 (6.9%) *E. coli* (18.7%)	- -	Zeryehun et al. (2013)
Jigjiga	Camel carcass PES Meat	2/70(2.86) 6/90(6.67) 4/70(5.71)	*E. coli* O157:H7	-	Henok (2014)

Table 3. Systematic summary of study done on foodborne *L .monocytogene* in Ethiopia

Location	Sample/source	Prevalence (%)	*Listeria* spp.	Antimicrobial susceptibility profile	References
A/A	Minced beef	29/61(47.5)	*L. monocytogene* (5.1%)	-	Molla et al., 2004
	Pork	37/53(69.8)	*L. innocua* (21.2%)		
	Chicken	8/52(15.4)	*L. seeligeri*		
	Fish	8/43(18.6)	*L. welshimeri*		
	Cottage cheese	1/61(1.6)	*L. murrayi*		
	Ice cream	20/46(43.5)	*L. grayi*		

Table 3. Contd.

A/A	Raw meat Raw milk Cottage cheese Cream cake	41/60(68.34) 6/60(10) 6/60(10) 13/60(21.67)	*L. monocytogene* (19.7%) *L. innocua* (39.4%) *L. seeligeri*(4.5%) *L. welshimeri* (12.12%) *L. murrayi* (13.6%) *L. grayi* (1.5%)	Firehiwot (2007)
A/A	Pasteurized milk Cheese Ice cream Cake Minced beef Pork Chicken carcass	0/101 0/102 43/101(42.7) 12/101(12.1) 48/102(47.7) 63/102(62.5) 16/102(16.67)	*L. monocytogene* (4.8%) *Listeria* spp. (21.8%)	Desalegn et al. (2009)
A/A	Liquid whole egg Raw beef Raw milk Cottage cheese	37/115(32.2) 39/76(51.3) 22/100(22) 4/100(4)	*L. monocytogene* (5.4%) *L. innocua* (15.9%) *L. seeligeri* (1%) *L. welshimeri* (1.8%) *L. murrayi* (0.8%) *L. grayi* (0.8%) *L. ivanovii* (0.5%)	Gebretsadik et al. (2011)
Jigjiga	Camel carcass PES Meat	0/70 0/90 1/70(1.43)	*L. monocytogenes*	Henok (2014)

Table 4. Systematic summary of studies conducted on foodborne *S. aureus* in Ethiopia

Location	Sample/source	Prevalence (%)	*Staphylococcus* spp.	Antimicrobial susceptibility profile	References (*=unpublished)
B/dar	Cow milk	147/1347(10.9)	*CNS* (49.6%) *S. aureus* (17.8%) *S. intermidius* (5.2%)	TTC, ERY, OXA, CHL, CL, S	Alemaw (2004)*
D/Zeit	Pasteurized milk Milk from Udder Bucket milk Stored milk	94/100(94) 70/77(91) 77/77(100) 12/12(100)	*S. aureus,* *S. intermidus,* *S. hyicus* *S. epidermidus*	-	Wubete (2004)
Adami- tulu	Goat milk	374/680(55)	*S. aureus* (12.8%) *CNS* (9.6%)	CLO, METH ,OTTC, ERY, CHL	Wakwoya et al. (2006)
D/Zeit	Cottage cheese Bucket milk Tank milk	48/200(24) 33/100(33) 46/100(46)	*S. aureus* (7%) *S. intermidius* (7%) *S. hyicus* (5%) *CNS* (12.8%)	-	Mekonnen (2009)

Table 4. Contd.

B/dar	Cow milk	99/139(71.2)	*S. aureus* (20.3%) *CNS* (51.9%)	-	Molalign et al. (2010)
Adama	Cow milk	59/140(42.14)	*S.aureus*	AMP (36.1%), STR (5.6%), PEN (94.4%), TMP-SULFA (58.3%)	Abera et al. (2010)
Jimma Shashemene	Kitifo Surface swab Carcass swab Cow milk	120 12 33 20/165(12.1) 217/364(59.6)	*S. aureus* (28.1%) Other *Staph* (22.1%)	(AMP, STR, AMC, ERY, OXA, VAN)	Haimanot et al. (2010) Desie et al. (2011)
Gondar	Cow milk	164/322(50.9)	*S.aureus* (16.5%) *CNS* (31.1%)	TTC,CAF, KAN, OXA, AMP, SU, S, ERY, CL	Nibret et al. (2011)
Jimma	Cow milk	164/218(75.22)	*S.aureus* (39.44) *CNS* (18.8%)	PEN-G, VAN, CHL,CAF, NAL, AMP	Tariku et al. (2011)
Yabello	Cow milk	577/712(81)	*S.aureus* (29.2%)	-	Adane et al. (2012)
Hawassa	Cow milk	78/160(48.75)	*S.aureus*	AMP, PEN-G, OXA	Dakaet al. (2012)
Humera & Abergelle	Goat milk Sheep milk	255 135 84/390(21.5)	*S.aureus* (27.7%) *CNS* (44.7%)	-	Gebrewahid et al. (2012)
Mekelle	Cow milk	128/174(73.56)	*S. aureus* (36%)	CHL, AMP, ERY, Trim-sulfa	Haftu et al. (2012)
Mekelle	Margarine Mayonnaise Sardine “wot” Macaroni “Feta “ “zahla” Mango juice Avocado Fruit mix Table scraping	2/10(20) 0/10 0/10 1/30(3.34) 1/10(10) 5/30(16.7) 2/10(20) 5/15(33.3) 2/17(13) 0/8 55/110(50)	*S. aureus*	-	Mekonnen et al. (2012a)
Mekelle	Meat Bucher shop Abattoir Street meat sale	2/30(6.7) 2/5(40) 3/5(60)	*S. aureus*	-	Mekonnen et al. (2012b)

Table 4. Contd.

A/A	Cow milk	71/146(64.54)	*S. aureus*(21.13%) *S. agalactiae* (18.3%) *CNS* (11.2%)	-	Abunna et al. (2013)
A/A	Cow milk	80/118(67.8)	*S. aureus*(28.7%)	-	Zeryehun et al. (2013)
Jigjiga	Camel carcass PES Meat	6/70(8.57) 29/90(32.22) 11/70(15.7)	*S. aureus*	-	Henok (2014)

Pen-G-penicillin G, S-streptomycin, CAF-chloramphenicol, tmp-sulfa-trimethoprim-sulfaoxizine, CL-clindamycine, Sul-sulfioxazole.

Table 5. Systematic summary on foodborne Campylobacteriosis in Ethiopia.

Location	Sample/source	Prevalence (%)	*Campylobacter* spp.	Antimicrobial susceptibility profile	References (*=unpublished)
A/A D/Zeit	Beef Sheep meat Goat meat Pork Chicken	14/227(6.2) 12/114(10.5) 7/92(7.6) 4/47(8.5) 13/60(21.7)	*C. jejuni* (78%) *C. coli* (18%) *C. lari* (4%)	-	Dadiand Asrat (2008)
D/Zeit	Sheep carcass	23/218(10.6)	*C. jejuni* (7.3%) *C. coli* (2.7%)	-	Woldemariam et al. (2009)
B/dar	Chicken	160/220(7.27)	*C. jejuni* (92.5%) *C. coli* (7.5%)	AMP, ERY, STR, TTC AMP, STR, TTC	Ewnetu and Mihret (2010)
Jigjiga	Camel carcass PES Meat	4/70(5.71) 3/90(3.33) 3/70(4.28)	*C. jejuni* (2.85%) *C. coli* (2.14%)		Henok (2014)

be important (Solomon and Hoover, 1999).

The pathogenesis of *C. gastroenteritis* is not fully characterized (Rollins and Joseph, 2001). A serious consequence of diarrheal diseases is the Guillain-Barrè syndrome (GBS) characterized by polyneuritis of the peripheral nerves, which may lead to either short-term or lengthy paralysis (Blaser et al., 1983).

In Ethiopia, few studies reported that *Campylobacter* species are common cause of childhood diarrhea and antimicrobial resistant strains were also reported (Beyene and Haile-Amlak, 2004). The absence of national surveillance program, limited routine culture availability for the isolation of *Campylobacter* species at clinical and research settings, the need for selective media and unique growth atmosphere; makes it difficult to give an accurate picture of the burden. This fact indicates that *Campylobacter* as a causative agent of diarrhea is not given appropriate weight and consideration in Ethiopia. Those studies which are done on foodborne Campylobacteriosis in different parts of Ethiopia are summarized in Table 5.

Very few published studies are found on foodborne campylobacteriosis in Ethiopia regardless of sever pathogenic cause of gastroenteritis in human. Studies from 2000-2014 show that only three published research were done in Addis Ababa and D/Zeit (Dadi and Asrat, 2008), D/Zeit (Woldemariam et al., 2009) and B/dar (Ewnetu and Mihretu, 2010) and one unpublished research done in Jigjiga (Henok, 2014). Since foodborne campylobactersis are the cause of diarrhea in human especially in children little emphases is given by human and veterinary medicine.

As a remark, researchers should give special attention to this area to assess and determine the prevalence; public health significance and antimicrobial susceptibility profile of foodborne campylobactersis with special emphases on *Campylobacter jejuni* and *Campylobacter coli*

in Ethiopia since this species become an emerging antimicrobial resistant strain due to consumption of not thoroughly cooked food of animal product like poultry since sometimes while cooking doro-wote when the chickens are young, the meat is easily cooked with minimum heat in this case some of the bacteria may survive heating temperature and transfer the antimicrobial resistant gene to the normal intestinal flora of human by either plasmid, transposons or transfor-mation.

All the published studies on common food borne pathogens such as *Salmonella* spp., *Escherichia coli* spp., *Listeria* spp., *Staphylococcus* spp. and *Campylobacter* spp. conducted by different investigators in Ethiopia have shown the widespread distributions of foodborne pathogen isolates in the community. Several common foodborne pathogens with their antimicrobial resistance profiles have been investigated from the year 2000-2014.

Recommendations

1. The epidemiology of foodborne pathogen in Ethiopia has not been well investigated and it requires continuous integrated surveillance both nationally and regionally in order to establish holistic figure for foodborne pathogen in the country.
2. The national research institutes and government universities should be able to identify foodborne pathogen to the level of serovar and measure quantitatively antibiotic susceptibility pattern, so that comparison with serovars isolated from humans, animals and food products could be possible. Additionally if all these institutions are working in well-organized way, it will avoid repeated work on same area and same pathogen finally saving extra costs for surveillance.
3. To decrease the incidence of foodborne pathogen in Ethiopia, besides giving attention in identification, susceptibility testing and reporting during routine bacteriological analysis, public health measures such as improving personnel, food hygiene and intensive health education should be implemented.
4. Finally, according to “publish or perish” motto of the scientific community, it is recommended that everyone should publish the research outputs and make them available to the public.

Conflict of interest

The author(s) have not declared any conflict of interests.

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Review

A review and future potential approach for *Campylobacter* control in retail poultry meats

Djamal Djenane[1]*, Javier Yangüela[2] and Pedro Roncalés[2]

[1]Université Mouloud MAMMERI. Département des Sciences Alimentaires. Faculté des Sciences Biologiques et des Sciences Agronomiques. Laboratoire de Qualité & Sécurité des Aliments. BP 17, 15000 Tizi-Ouzou, Algeria.
[2]Departamento de Producción Animal y Ciencia de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, C/Miguel Servet, 177-50013 Zaragoza, Spain.

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Campylobacteriosis is considered the most frequent zoonosis in humans, and the handling and/or consumption of poultry meat are considered the main source for human infection. Moreover, largely owing to the recent food authority ban on the use of antibiotic growth promoters in animal feed, it is now very important to look for new effective strategies to reduce the incidence of these bacteria in the host. Chicken intestines, and also the intestines of other animals, are the only sites where *Campylobacter* proliferates in meat. Therefore, the development of a novel approach for controlling *Campylobacter* could be a very valuable alternative strategy in the fight to eliminate these bacteria from the poultry meat chain.

Key words: Poultry, meat, retail, *Campylobacter*, control, novel approach.

INTRODUCTION

Campylobacter contamination of poultry carcasses is common, and chicken are generally recognised to play a significant role in human *Campylobacter* infection (Raut et al., 2012; Torralbo et al., 2014). Campylobacteriosis remains the most frequently reported zoonotic disease in humans in the European Union (Table 1). It is estimated that there are approximately nine million cases of human campylobacteriosis per year in the EU 27 (EFSA, 2010, 2011; Kvalsvig et al., 2014).

Some studies show that more than 98% of products derived from raw chicken in shops could be contaminated with this bacterium (Jacobs-Reitsma et al., 2008). *Campylobacter* are ubiquitous bacteria, frequently found in the alimentary tracts of animals, especially birds and commonly contaminate the environment, including water (Figure 1).

Campylobacteriosis in humans is caused by emerged thermotolerant *Campylobacter* spp. these pathogens are a leading cause of zoonotic enteric infections in most developed and developing nations worldwide. *Campylobacter jejuni* has recently overtaken *Salmonella* spp. as the major reported source of food-borne bacterial diseases within the European Union (Table 2).

A number of countries have instituted successful

*Corresponding author. E-mail: djenane6@yahoo.es, d.djenane@hotmail.com. Tel: 00 (+213) 779 001 384. Fax: 00 (+213) 26 21 68 19.

Table 1. Reported campylobacteriosis confirmed in humans (EFSA, 2010, 2011).

Country	Cases/100,000 inhabitants
Austria	51.4
Belgium	47.9
Bulgaria	0.2
Cyprus	2.9
Czech Republic	193.3
Denmark	63.4
Estonia	11.5
Finland	84.0
France	5.4
Germany	78.9
Hungary	54.7
Ireland	39.8
Italy	0.4
Latvia	0.0
Lithuania	22.5
Luxembourg	90.7
Malta	18.8
Poland	0.7
Romania	0.1
Slovenia	44.2
Spain	11.4
Sweden	83.8
The Netherlands	39.2
The United Kingdom	90.9

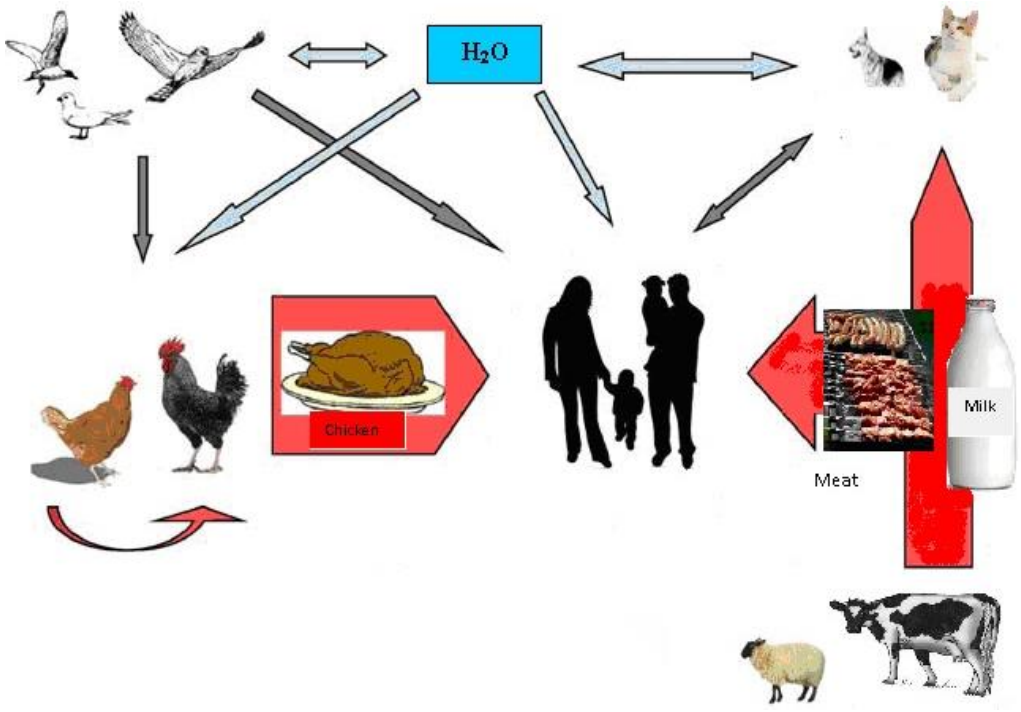

Figure 1. *Campylobacter* spp. sources and risk factors for human illness.

Table 2. Prevalence of *Campylobacter*-contaminated broiler carcasses in the EU (EFSA, 2010).

Country	Prevalence (%)
Austria	47.8
Belgium	31.0
Bulgaria	29.6
Cyprus	30.6
Czech Republic	61.3
Denmark	19.0
Estonia	2.0
Finland	3.9
France	76.1
Germany	48.9
Hungary	50.1
Iceland	25.0
Ireland	83.1
Italy	63.3
Latvia	41.0
Lithuania	41.5
Luxembourg	100
Malta	96.8
Norway	3.2
Poland	78.9
Portugal	82.0
Romania	77.0
Slovenia	78.2
Spain	88.0
Sweden	13.2
Switzerland	59.0
The Netherlands	24.4
The United Kingdom	75.3

prevention and surveillance measures against *Campylobacter* infections. However, campylobacteriosis is challenging to study and some aspects remain poorly understood (Kvalsvig et al., 2014; Macritchie et al., 2014). *C*. *jejuni* has been found to be associated with biofilms of other bacterial species. Biofilm formation may play a role in the epidemiology of *C*. *jejuni* infections (Gunther and Chen, 2009). Although it is generally recognized that there are many sources of *Campylobacter* spp., campylobacteriosis is predominantly believed to be associated with the consumption of poultry meat, especially fresh broiler meat (Table 3).

Over the past decade, risk analysis, a process consisting of risk assessment, risk management and risk communication, has emerged as a structured model for improving food control systems, with the objectives of producing safer food and reducing the numbers of food-borne illnesses (Milios et al., 2014). Therefore, control of *Campylobacter* spp. commonly focuses on reducing the occurrence of *Campylobacter* in broiler meat. In recent years, several quantitative risk assessments for *Campylobacter* in broiler meat have been developed to support risk managers in controlling this pathogen (Comin et al., 2014). The risk assessments are not only used to assess the human incidence of campylobacteriosis due to contaminated broiler meat, but more importantly for analyses of the effects of control measures at different stages in the broiler meat production chain. Microbiological risk assessment can be considered as a tool that can be used in the management of the risks posed by food-borne pathogens and in the elaboration of standards for food in international trade. Given the public health and economic problem represented by *Campylobacter*, it is important to take measures in order to reduce *Campylobacter* prevalence throughout the poultry production chain leading to a reduced incidence of the human illness. Several strategies have been applied to reduce *Campylobacter* counts on chicken meat, including attempts to eliminate *Campylobacter* from the farms by increasing biosecurity and the separation of contaminated flocks, and by improving hygiene during the process of slaughtering (Sasaki et al., 2014). In addition, several experimental approaches like the reduction of colonization by competitive exclusion, antibacterial agents, or phage therapy are being investigated for their efficacy (Timms et al., 2010). The combination of prebiotics and probiotics to reduce *Campylobacter* are known as symbiotic, and may have antimicrobial activity (Klewicki and Klewicka, 2004). It is generally acknowledged that *Campylobacter* is sensitive to acid conditions. Several strategies developed to reduce *Campylobacter* populations are based on the acidification of the pathogen environment or by acidification of drinking water and feed (Chaveerach et al., 2002). Although these measures undoubtedly will help to control shedding of *Campylobacter* by the animals and may reduce the number of positive flocks, vaccination of poultry against *Campylobacter* will probably be the most effective and remains a major goal. However, several studies have actually pointed out partial association between the veterinary use of antibiotics and the emergence of resistant strains of *Campylobacter* related to human enteritis (Luangtongkum et al., 2006).

In recent years, there has been increased research interest in the use of no thermal alternative methods for microbial inactivation, such as, high hydrostatic pressure or pulsed electric fields. The attraction of these technologies lies in the production of microbiologically safe foods with minimal changes in their sensory and nutritional attributes. Several relatively recent studies describe in detail the antimicrobial properties of wine against *C*. *jejuni*. The results indicate that the exposure of contaminated food to wine, as in marinade conditions, significantly reduces the number of viable cells of *C*. *jejuni* (Isohanni et al., 2010). Consumers demand high quality, natural, nutritious, fresh appearance and convenient meat products with natural flavour and taste

Table 3. Risk factors associated with enteric *Campylobacter*.

Risk factors	References
Drinking untreated water, drinking raw milk, eating undercooked chicken, cat in household.	Hopkins et al. (1984)
Eating undercooked chicken, eating pre-packed sandwiches, consumption of raw milk, consumption of mushrooms.	Harris et al. (1986)
Eating undercooked chicken, daily contact with cat, consumption of raw milk.	Deming et al. (1987; Schmid et al. (1987)
Eating undercooked chicken.	Kapperud et al. (1992)
Eating undercooked chicken, use of untreated water.	Ikram et al. (1994)
Travel abroad, eating undercooked chicken.	Schorr et al. (1994)
Handling raw meat, contact with animals with diarrhoea, consumption of untreated water.	Adak et al. (1995)
Eating undercooked chicken and consumption of chicken prepared at restaurant, travel abroad, Drinking unpasteurized milk, contact with animals.	Eberhart-Phillips et al. (1997)
Foreign travel, eating chicken, drinking milk from bottles damaged by birds, consumption of medication Omeprazole.	Neal and Slack (1997)
Drinking unpasteurized milk, consumption of chicken, consumption of pork with bones, barbecuing, daily contact with chickens, living or working on a farm.	Studahl and Andersson (2000)
Eating chicken prepared commercially	Effler et al. (2001)
Travel abroad, eating chicken prepared commercially	Rodrigues et al. (2001)
Consumption of undercooked poultry, consumption of red meat at a barbecue, Consumption of grapes. Drinking unpasteurized milk. Travelling abroad.	Neimann et al. (2003)
Consumption of untreated water, consumption of undercooked poultry. Contact with poultry.	Kapperud et al. (2003)
Contact with poultry	Potter et al. (2003)
Travel abroad, Consumption of chicken prepared at restaurant (and other meats).	Friedman et al. (2004)
Food handling and consumption of undercooked problematic chickens.	Osiriphun et al. (2012)
Eating roasted chicken meat and Russian salad.	Calciati et al. (2012);
Consumption of salad.	Signorini et al. (2013)

Table 4. Growth characteristics of thermophilic *Campylobacter* species (Park, 2002).

	Optimum	Inhibition
Temperature	40–42°C	< 30°C – > 45°C
pH	6.5–7.5	< 4.9 – > 9.0
O_2	3–5%	0 – 15 to 19%
CO_2	10%	–
N_2	85%	–
Water activity	0.997	< 0.987
NaCl	0.5%	> 2 %

and an extended shelf-life. One area of research is the development of new and improved methods of meat preservation. Due to negative consumer perceptions of artificial preservatives, attention is shifting towards alternatives that the consumers perceive as natural and in particular, bio preservation and plant extracts, including their essential oils (EOs) and essences. It is well established that these natural compounds have antimicrobial properties against the human enteropathogen *C*. *jejuni*.

This paper presents the short review of recent works on the strategies application to prevent or reduce *Campylobacter* contamination in poultry meat.

CAMPYLOBACTER

Campylobacter cells are Gram-negative spirally curved rods. In general, *Campylobacter* species do not grow in conventional aerobic or anaerobic culture systems. *Campylobacter* are O_2-sensitive micro-aerophilic bacteria (Table 4), with optimal growth in an atmosphere containing 5-10% O_2 and 1-10% CO_2, which is related to its niche in the avian tract (Park, 2002). They do not ferment or oxidize sugars and are sensitive to hydrogen peroxide and superoxide anions produced in media. *C*. *jejuni* and *C. coli* are distinguished from most other *Campylobacter* species by their high optimum growth temperature (42°C). The *Campylobacter* genus has 17 species, 14 of

Table 5. Prevalence (%) and species of *Campylobacter* in retail meat under modified atmosphere packaging (MAP) or unpackaged (Lynch et al., 2011).

Meat	Packaging	No. of samples	Positive (%)
Beef	MAP	92	36 (39)
	Unpackaged	94	30 (32)
subtotal		186	66 (36)
Pork	MAP	91	21 (23)
	Unpackaged	88	19 (22)
subtotal		179	40 (22)
Chicken	MAP	55	9 (16)
	Unpackaged	130	21 (16)
subtotal		185	30 (16)

which have been associated with human illnesses, and of these, *C*. *jejuni* and *C. coli* cause more than 95% of the infections attributed to this genus (Park, 2002).

This combination of strict requirements places *C*. *jejuni* in the unique group of food-borne pathogens which are not able to multiply outside of the host and grow in food during either processing or storage. The bacterial cells react to temperature downshift by altering cell morphology and physiology. As the temperature decreases, coccoid cells are formed, resulting in viable but non-cultivable forms. This is considered to be an adaptive response to hostile external environments (ICMSF, 1996). The resuscitation of non-cultivable cells has been demonstrated in chickens (Stern et al., 1994). Even though *C*. *jejuni* does not grow below 30°C, the bacterium survives on raw meat surfaces at refrigerated temperatures and thus poses a risk to the consumer (Ligowska et al., 2011). Superoxide dismutase plays an active role in the protection against oxidative stress and aerotolerance and is an important factor for survival of *Campylobacter* in food (Park, 2002). *Campylobacter* are particularly sensitive to drying and reduced pH. In addition, *Campylobacter* is sensitive to salt concentrations above 1.5%. *C*. *jejuni* and *C*. *coli* are sensitive to heat and do not survive cooking or pasteurization temperatures with D-values of 0.21–2.25 min at 55-60°C (ICMSF, 1996).

ANTIBIOTIC-RESISTANCE

The use of antimicrobial agents in food animals has resulted in the emergence and dissemination of antimicrobial-resistant bacteria, including antimicrobial-resistant *Campylobacter*, which has potentially serious impact on food safety in both veterinary and human health (Messad et al., 2014; Abay et al., 2014). The antimicrobial resistance increased, especially to a fluoroquinolone, ciprofloxacin, in many *Campylobacter* species (Cakmak and Erol, 2012; Lazou et al., 2014). This is particularly seen as a risk for fluoroquinolone resistant *Campylobacter* (Geenen et al., 2010), and the use of antimicrobials to control *Campylobacter* in broilers is strongly discouraged. Andersen et al. (2006) found that raw food samples from the retail level represent an important sampling point, which reflects the consumer exposure to resistant *C*. *jejuni* originating from raw poultry.

RETAIL POULTRY MEATS

Many papers have reported on the level of contamination with *Campylobacter* spp. in retail poultry meats and/or by-products (Table 5). For example, the prevalence of *Campylobacter* spp. was reported to be 32.0-43.0% in Germany (Adam et al., 2006), 50.5-73.5% in the UK (Meldrum et al., 2006), 79.0% in the USA (Nannapaneni et al., 2005), 62.4% in Canada (Valdivieso-Garcia et al., 2007) and 62.9% in southern Spain (Torralbo et al., 2014).

The majority of *Campylobacter* infections are acquired via the oral route after handling raw poultry or consuming undercooked poultry. Seasonality has been found to influence the *Campylobacter* prevalence in retail chicken meat (Boysen et al., 2011; Cakmak and Erol, 2012). *Campylobacter* contamination in chicken is highest during summer and early autumn. In the home, during meal preparation, individuals can be exposed to *Campylobacter* from fresh chicken through a large number of pathways. These pathways could include: direct contamination from the chicken to any food commodities not undergoing a subsequent cooking step before ingestion; indirect contamination of surfaces upon which cooked products or ready-to-eat food are placed; contamination directly onto hands and subsequent ingestion; insufficient cooking; and a wide variety of other potential contamination events. Transfer can be facilitated by liquid carried on hands, utensils and cutting boards and these mechanisms may be a significant contributor to exposure and food-borne illness. Unsafe food handling procedures in private kitchens are assumed to be responsible for a large number of cases of food-borne diseases in most countries (Zhao et al., 1998). Lynch et al. (2011) demonstrate that retail meats contain a much more diverse range of *Campylobacter*, particularly on beef and pork products. The incidence of Campylobacters on beef (36%) was significantly higher than on pork (22%) or chicken (16%), and far exceeds previously reported prevalence levels.

It has been found that polyphosphates present in exudates processed chicken, were determined to be largely responsible for the improved survival of *Campylobacter* spp. Therefore, polyphosphates used to enhance chicken quality aid in sustaining the numbers of *Campylobacter* bacteria, increasing the opportunity for disease via cross-contamination or improperly cooked poultry (Nereus and Gunther, 2010). Organic and other

no conventional broiler products are now readily available for retail in many countries, yet very little is known about the status of these broiler flocks with regard to the prevalence of *Campylobacter*.

NEW DEVELOPING STRATEGIES AGAINST *CAMPYLOBACTER*

Primary production

This involves feeding with complex mixtures of bacteria that reduce attachment of pathogens to the gut mucosa. Competitive exclusion flora is a concept taking advantage of bacterial antagonism to reduce animal intestinal colonization by pathogenic microorganisms (Schneitz, 2005). Commensal gut flora may be manipulated by changing the diet of the animal and some research has shown that chickens given certain diets are better able to resist challenge with campylobacters.

Bacteriophage therapy is one possible means by which the colonization could be controlled, thus limiting the entry of campylobacters into the human food chain (Carrillo et al., 2011). Similarly, experiments suggest that treating live birds with specific bacteriophages shortly prior to slaughter may be an effective control measure (Havelaar et al., 2007). There has been a renewed interest in the use of bacteriophages as ‘‘therapeutic’’ agents; a prerequisite for their use in such therapies is a thorough understanding of their genetic complement, genome stability and their ecology to avoid the dissemination or mobilisation of phage or bacterial virulence and toxin genes (Timms et al., 2010).

Other method to reduce the *Campylobacter* load in poultry is the use of bacteriocins from bacteria as a therapeutic treatment for chickens colonized by *Campylobacter*. Svetoch and Stern (2010) reviewed bacteriocin application to reduce the cecal *Campylobacter* counts in broiler chickens of colonized flocks. By feeding the animals therapeutic feed at the appropriate moment in the cycle, levels and frequency of colonization can be reduced, which may be effective in lowering the human health risk imposed by *Campylobacter*. Lin (2009) has reviewed anti-*Campylobacter* bacteriocins for potential use in reducing the numbers of *Campylobacter* (*jejuni* as well as *coli*) in poultry. Stern et al. (2006) found that control chickens (standard feed) were colonized in the caecum with 6.6-8.3 $\log_{10}$ cfu/g of *Campylobacter*, while all treated chickens (feed modified with purified bacteriocin) contained undetectable numbers (< 2 $\log_{10}$ cfu/g). Svetoch et al. (2008) administered bacteriocin to young chicks. High levels of *C*. *jejuni* were found in the control chicks (8.40 $\log_{10}$ cfu/g of caecal contents), while no *Campylobacter* was detected in the treated group. Thus, it seems that bacteriocins, administered just before slaughter, can reduce *Campylobacter* colonization in the chicken caecum to undetectable levels.

Supplementing bacteriocin in drinking water at 3.5-25 mg per bird for three days before slaughter was most effective, resulting in a complete elimination of *C*. *jejuni* in 90% of the cases. The safety of these bacteriocins was confirmed by conducting experiments on monkey and human cell cultures as well as in treated mice and chickens.

Orally given probiotic bacteria could prevent colonisation of chicken with pathogenic *Campylobacter* (Morishita et al., 1997). Chaveerach et al. (2004) found that *Lactobacillus* (P93) strain isolated from conventional chicken had potential inhibitory activities against all tested *Campylobacter*. Probiotics can be incorporated in the diet. This is based on feeding with viable microorganisms antagonistic toward pathogens via either modifying environmental factors in the gut or producing antimicrobial compounds (Morishita et al., 1997). Santini et al. (2010) reported both marked *in vitro* and *in vivo* activity for *Bifidobacterium longum* towards *Campylobacter*. Recently, Wang et al. (2014) suggested that *Lactobacillus* strains N8, N9, ZL4 and ZL5 could be used as potential probiotics in food applications against *C*. *jejuni* infection.

With the ban of dietary antimicrobial agents, the use of probiotics, prebiotics and synbiotics has attracted a great deal of attention in order to improve intestinal health and control food-borne pathogens, which is an important concern for the production of safe meat and meat products. Combinations of prebiotics and probiotics are known as synbiotics, and may have antimicrobial activity (Klewicki and Klewicka, 2004). Fooks and Gibson (2002) have yet recorded a *C*. *jejuni* inhibition *in vitro*, with a population reduction below detectable level after 24 h culture, with a *Lactobacillus plantarum* or *Bifidobacterium bifidum*, when combined with oligofructose or an oligosaccharide. Finally, addition of mannanoligosaccharide to the feed of naturally infected birds and xylanase to the feed of artificially infected broilers, as prebiotics, resulted both in a minor, although significant decrease in cecal *C*. *jejuni* counts in these animals (Baurhoo et al., 2009). The study of Baffoni et al. (2012) highlighted the positive effect of the synbiotic approach for *C*. *jejuni* reduction in broiler chickens, which is of fundamental importance for the safety of poultry meat consumers. The galactooligosaccharide was then combined with a probiotic *Bifidobacterium* strain (*Bifidobacterium longum* subsp. *longum* PCB133), possessing antimicrobial activity against *C*. *jejuni*.

In chicken meat

Reducing human *Campylobacter* infection cases has become a priority for the UE Governments. However, the public's views on acceptability of interventions to reduce *Campylobacter* in poultry production are poorly understood

in the UE and in other countries around the world. Overall, findings indicate that increasing consumer acceptability of the most effective interventions is likely to be a difficult process (Macritchie et al., 2014).

Nonthermal methods

In recent years, there has been increased research interest in the use of nonthermal alternative methods for microbial inactivation, such as, high hydrostatic pressure or pulsed electric fields. The attraction of these technologies lies in the production of microbiologically safe foods with minimal changes in their sensory and nutritional attributes. Sagarzazu et al. (2010) showed that incu-bation of heat-treated cells in the presence of sodium pyruvate highly improved the survival ability of *C*. *jejuni*; on the contrary, it did not enhance survival ability of this microorganism after exposure to pulsed electric fields treatments.

Irradiation

Haughton et al. (2012) found that exposure of skinless chicken fillet to near ultraviolet/visible light (NUV–vis light: 395±5 nm) for 1 or 5 min at 3 cm distance reduced *C*. *jejuni* by 2.21 and 2.62 $\log_{10}$ cfu/g, respectively. Chun et al. (2010) investigated the applicability of UV-C irradiation (wavelengths of 220-300 nm) on the inactivation of *C*. *jejuni* in ready-to-eat meat and poultry meat respectively, the results have clearly indicated that UV-C irradiation effectively decreased *C*. *jejuni* inoculated on meat during storage. Irradiation of food materials, using electron beams (from electron accelerators) or high-energy electromagnetic radiation (gamma-rays from ^{60}Co or X-rays), is permitted in some European countries and will kill campylobacters and other infectious bacteria (Sparks, 2009). The application of irradiation in poultry at doses of 1-10 kGy eliminates pathogenic bacteria (Lacroix and Ouattara, 2000). Raut et al. (2012) found that radiation treatment with a dose of 1 kGy could achieve complete elimination of 10^5 cfu of *Campylobacter*/g in poultry meat samples. However, irradiation might have some effects on organoleptic quality of meat products. The threshold dose above which off-flavors are detected in irradiated meats was reported to be 2.5 kGy for poultry (Hanis et al., 1989). Natural antioxidants from spices could be employed to stabilize fats and control oxidative deterioration of foods during irradiation. The effect of the combination of irradiation and marinating with rosemary and thyme extract on the sensitivity of pathogen and organoleptic characteristics of poultry has also been investigated. A dose of 2-3 kGy would be sufficient to decontaminate meat from campylobacters (Ingram and Farkas, 1977; Monk et al., 1995). However, application of this technology has been very limited. A disadvantage in the European Union is that at present use of gamma-irradiation for meat is strongly discouraged. Its limited use appears to be due to distrust by the public of any process which depends on the nuclear industry as well as lack of knowledge by the public in general concerning food borne infections and the effectiveness of irradiation. A preferred option might be to use electron accelerators which require no isotope. These are used, particularly in UE, to decontaminate raw chicken portions (Carry et al., 1995). Kampelmacher (1984) showed that a dose as low as 1 kGy was effective in reducing *C*. *jejuni* by more than 4 log-cycles with this dose. The directive 1999/3/EC contains a list of foodstuffs authorized for irradiation treatment and the doses allowed. So far, only dried aromatic herbs, spices and vegetable seasonings are included in the list. However, irradiation of other foodstuffs including poultry is temporarily permitted in some Member States. In the United States, FDA and USDA have approved irradiation of poultry meat at a maximum dose of 3 kGy to control foodborne pathogens such as *Campylobacter* (Keener et al., 2004).

Essential oils

Increased consumer demand for all natural food products has put pressure on industry and regulatory agencies to closely examine the potential for use of natural antimicrobials that prevent or control the growth of food-borne pathogens and spoilage microorganisms. Although many studies have indicated that EOs has the potential to be used as a natural antimicrobial preservative in meats (Djenane et al., 2011a, b; 2012a, b), the success in simple agar diffusion systems has not been seen in foods because the antimicrobial activity of EO is reduced in the presence of fat and protein (Burt, 2004). It is generally supposed that the high levels of fat and/or protein in foodstuffs protect the bacteria from the action of the EO in some ways (Tassou et al., 1995). In one of such study, an increase in concentration of 10-fold when used in pork sausages, 50- fold when used in soup and 25 to 100-fold when used in soft cheese, 2-fold when used in minced beef and chicken was required to produce a similar effect to that reported *in vitro* (Djenane et al., 2011a, 2012b; Tassou and Nychas, 1996). Also, the oils may have been less effective on the chicken skin because of the rough surface of the skin, which allowed for greater adhesion by the bacteria (Fisher and Phillips, 2006). EOs, as antimicrobial agents, present two main characteristics: the first is their natural origin which means more safety for consumers and the second is that they are considered to be low risk for resistance development by pathogenic microorganisms. Kurekci et al. (2013) found that EOs and related terpenoid compounds can have strong anti-*Campylobacter* activity without adversely affecting the fermentation potential of the chicken-caeca microbiota. EOs and their active compounds may have the potential

Table 6. Effect of different phenolic compounds present in wine on the viability of *C*. *jejuni* using concentration of 1000 mg/L for n = 4 (+ indicates a significant difference with respect to control) (Gañan et al., 2009).

Phenolic compound	Concentration (mg/L) = 1000
Caffeic acid	+
Catechin	-
Cumaric acid	+
Epicatechin	+
Ferulic acid	+
Gallic acid	+
Methyl gallate	+
p-Hydroxybenzoic acid	+
Quercetin	-
Synaptic acid	+
Tryptophol	+
Vanillic acid	+

to control *C*. *jejuni* colonisation and abundance in poultry.

In vitro studies have demonstrated the efficacy of different natural substances such as the EOs of cedar wood, jasmine, marigold, ginger, patchouli, carrot seeds, celery, spikenard (Friedman et al., 2002) and orange (Nannapaneni et al., 2009) as antimicrobial compounds with activity against some strains of *C*. *jejuni*. However, they have not yet been demonstrated to effectively control this pathogen in chickens. Coriander EO was tested *in vitro* for antimicrobial activities against *C*. *jejuni* using disk diffusion and minimal inhibitory concentration determination assays, it has been noted that coriander oil exhibited the strongest antimicrobial activity against tested *C*. *jejuni*. In evaluating the antimicrobial potency of coriander oil against *C*. *jejuni* on chicken meat, it was found that the oil at concentration of 0.5% v/w killed all the bacteria on the meat, while 0.1 and 0.25% v/w oils reduced the bacterial cell loads on the meat from 5 to 3 and 1 log cfu/mL, respectively (Rattanachaikunsopon and Phumkhachorn, 2010).

Antimicrobial activities of the EOs of various herbs were investigated by Abdollah et al. (2010) against *C*. *jejuni* and *Campylobacter coli* isolated from chicken meat. The results indicated that the EO of these plants displayed remarkable activity against *C*. *jejuni* and *C*. *coli* and, therefore, they could be used as natural anti-*Campylobacter* additives in meat. Several recent studies described in detail the antimicrobial properties of some EOs against *C*. *jejuni*, which may be envisaged as natural alternatives to chemical-based antibacterial for food safety and preservation (Bakkali et al., 2008; Solomakos et al., 2008; Djenane et al., 2011a, 2012a). Despite the potential of many common plants and EOs is considerable, knowledge of this area and studies on their biological activities remain scarce. Most of the data published on the antimicrobial properties of plant EOs are fragmented and employ only basic screening techniques. Moreover, most studies on the antimicrobial action of plant extracts have been conducted *in vitro*, so that little information exists regarding the antimicrobial activity of EOs in food systems. By using disc diffusion assay, Wannissorn et al. (2005) and Djenane et al. (2012b) evaluated the antimicrobial activity of various EO samples extracted from various plants against *C*. *jejuni*. Tested EOs showed promising antibacterial activity against target bacteria. Djenane et al. (2012b) support the possible use of *Inula graveolens*, *Laurus nobilis*, *Pistacia lentiscus* and *Satureja montana* EOs, particularly that from *I*. *graveolens*, for the preservation of chicken meat. By using the described method, chicken meat can be stored in a modified atmosphere assuring a low risk associated with *Campylobacter*, at the same time that lipid oxidation is inhibited, giving rise to a higher sensory quality. The ability of *I*. *graveolens* to inhibit *C*. *jejuni*, which are Gram-negative bacteria, makes it more interesting for use to prevent food-related illness caused by other Gram-negative bacteria. Aslim and Yucel (2008) found that the EO obtained from *Origanum minutiflorum* showed strong antimicrobial activity against all the tested ciprofloxacin-resistant *Campylobacter* spp. It also suggests that the EO of *O*. *minutiflorum* may be used as a natural preservative in food against food-borne disease, such as Campylobacteriosis. Many studies have demonstrated that higher concentrations of EOs are required in food systems than *in vitro* investigations (Djenane et al., 2011a, 2012b). The use of EO vapours may be a potential way of combating the organoleptic effect brought about by direct contact between the food and EO. However, longer exposure to the vapour is required to produce a similar inhibitory effect (18 h as against 60 s) which has cost implications for the food industry (Fisher and Phillips, 2006).

Grape seed extract and wine

Silván et al. (2013) investigated the effects of grape seed extract on the inactivation of *C*. *jejuni*, the results have clearly indicated that the antibacterial activity against *C*. *jejuni* of the collected fractions showed that phenolic acids, catechins and proanthocyanidins were mainly responsible for the behaviour observed. Isohanni et al. (2010) suggested that wines could be used as antimicrobial ingredients together with the addition of further antimicrobial agents in meat marinades to reduce the numbers of *Campylobacter* in naturally contaminated poultry products, thus lowering the risk of *Campylobacter* cross-contamination and transmission through food. According to Gañan et al. (2009), wine constitutes an adverse environment for the survival of *C*. *jejuni* (Table 6). Furthermore, it would be interesting to study the possible use of phenolic compounds in wine as an alternative to the use of antimicrobial growth promoters against these bacteria in broilers.

Active packaging

Interest in the use of active packaging systems for meat and meat products has increased in recent years (Kerry et al., 2006). Changes in consumer preferences have led to innovations and developments in new packaging technologies. Active packaging is useful for extending the shelf life of fresh, cooked and other meat products. Forms of active packaging relevant to muscle foods include oxygen scavengers, carbon dioxide scavengers and emitters, drip absorbent sheets, antioxidant and antimicrobial packaging (Camo et al., 2011). Sánchez-González et al. (2011) found that antimicrobial films were prepared by incorporating different concentrations of various EOs, into chitosan and hydroxypropylmethyl-cellulose films. Their antibacterial effectiveness against pathogens bacteria was studied at 10°C during a storage period of 12 days. Hydroxypropylmethylcellulose-EO and chitosan-EO composite films presented a significant antimicrobial activity against the pathogens considered.

Combined methods

Study of Smigic et al. (2010) highlighted the importance of combining decontamination technologies with subsequent storage under O_2-rich atmosphere, at low pH and low temperature to the control survival and growth of *C*. *jejuni*. The combination of heat and acid pH was one of the first combined processes used by the food industry, with the objective of reducing the intensity of heat treatments. This practice has the advantage of decreasing heat resistance of *C*. *jejuni*, but also of preventing the growth of survivors (Palop et al., 1999). Gálvez et al. (2010) found that application of natural antimicrobial substances (such as bacteriocins) combined with novel technologies provides new opportunities for the control of pathogenic bacteria, improving food safety and quality. Bacteriocin-activated films and/or in combination with food processing technologies (high-hydrostatic pressure, high-pressure homogenization, in-package pasteurization, food irradiation, pulsed electric fields, or pulsed light) may increase microbial inactivation and avoid food cross-contamination. Piskernik et al. (2011) found the synergistic effect of freezing and rosemary extract antimicrobial activity. The combination of pre-freezing and plant extract treatment reduced the *C*. *jejuni* cell number by more than 2.0 log reduction.

CONCLUSION

Campylobacteriosis is considered the most frequent zoonosis, and the handling and/or consumption of chicken meat is considered the main source for human infection. The reduction of the rates of infection in chickens should make an effective contribution to substantially controlling the illness in humans. However, the increase of the general concern about the spreading of antibiotic resistance in humans has determined the elimination of antibiotics as growth promoters in livestock. At this point, it is essential to search for new, natural and sustainable strategies to reduce the incidence of this bacterium in poultry meat. Since chicken intestines, and also the intestines of other animals, are the only sites where *Campylobacter* proliferates in the food chain, it is essential to control the pathogen at these locations. The solution to the problem of *Campylobacter*-contaminated chicken by developing strategies must be economically viable, sustainable and legal, as well as acceptable to the consumer.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Review

Environmental stress conditions affecting the N_2 fixing *Rhizobium*-legume symbiosis and adaptation mechanisms

Sara LEBRAZI and Kawtar FIKRI BENBRAHIM*

Laboratory of Microbial Biotechnology, Faculty of Sciences and Technology, Sidi Mohamed Ben Abdellah University, P. O. Box 2202, Imouzzer Road, Fez, Morocco.

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Rhizobia are bacteria which fix atmospheric nitrogen in association within the root or the stem nodules of legume plants and transform atmospheric nitrogen to ammonia. Biological nitrogen fixation is an important process for sustainable land management, because nitrogen is the principal crop production's limiting factor. However, several environmental conditions such as salinity, temperature, acidity/alkalinity, drought, heavy metals, etc., are critical factors which can have detrimental effects on the steps involved in *Rhizobium*-legume symbiosis as infection process, nodule's development and function, resulting in low nitrogen fixation and crop yield. The presence of *Rhizobium*- legume symbioses able to fix appreciable N_2 amounts under unfavorable conditions is very interesting, because these symbioses represent the best source of nitrogen especially in arid and semi-arid regions, where they contribute to land stabilization and fertilization. Hence, the better understanding of rhizobial physiological responses to different intrinsic and extrinsic stresses factors is very important to improve crop production by harnessing biological nitrogen fixation process.

Key words: *Rhizobium*, legume, symbiosis, environmental stress, heavy metals, soil fertility.

INTRODUCTION

Nitrogen is a major limiting factor in agricultural production even if it represents almost 80% of the atmosphere (Abd-Alla et al., 2014). This paradox is due to the high stability of the nitrogen molecule (N_2) and to the fact that only some prokaryotic organisms are able to reduce it in an available form.

The biological nitrogen fixation (BNF) is a natural phenomenon consisting on the conversion of atmospheric nitrogen into ammonia by the nitrogenase enzyme complex. This biological reduction of N_2 to NH_3 is a highly endergonic process with a minimum energy requirement of Ca.960 KJ mol^{-1} N-fixed (Sprent and Raven, 1985). Nitrogenase function requires ATP and electrons, supplied respectively by respiration and electron carriers, usually ferrodoxin. Nitrogenase catalyzes the reduction of several substrates, including H^+, N_2 and C_2H_2. The principal

*Corresponding author. E-mail: kawtar.fikribenbrahim@usmba.ac.ma or kawtarbenbrahim@hotmail.com.

reaction for dinitrogen reaction is as follows:

$$N_2 + 16\ MgATP + 8\ e^- + 8\ H^+ \longrightarrow 2\ NH_3 + H_2 + 16\ MgADP + 16\ Pi$$

The energy requirements in this symbiosis are provided to bacteria by carbonaceous substances resulting from plant photosynthesis. *Rhizobium* can infect some root cortex cells of leguminous plants and initiate the formation of a new plant organ, the root nodule. These bacteria proliferate within root nodule cells then differentiate into a nitrogen fixing form called a bacteroid, which can fix the atmospheric nitrogen (Chanway et al., 2014).

The *Rhizobium*-legume symbiosis presents many advantages for both host plant and rhizobial bacteria by stimulating plant growth in nitrogen-deficient soils, offering the major success factor of the legume's family as compared to other plants and offering an adequate bacterial microhabitat necessary for nitrogen fixation (Noel, 2009).

Furthermore, this symbiosis is the result of a balance between environmental factors affecting both plant and bacteria. So the success of the legumes infection and nodulation depends on environment factors and *Rhizobium* survival. Environmental stress impose a major threat to both symbiotic nitrogen fixation and agriculture which can be limited by soil and climatic factors such as salinity, drought and temperature. For this reason, the *Rhizobium*'s tolerance to different environmental stresses is a desired property for use in nitrogen-depleted soils.

This review is focused on the study of the physiological responses to different stresses factors that can affect the rhizobial survival and the symbiotic nitrogen fixation in a perspective to understand the limiting factors of this symbiotic association and to better harness this biological process.

STRESS FACTORS AFFECTING SYMBIOSIS AND NITROGEN FIXATION

Various factors such as the soil physico-chemical composition can interfere with the infection process and nodulation, or can influence the activity of nitrogen-fixation during the symbiosis (Kinkema et al., 2006).

Salt and osmotic stress

Salinity is one of the major factors threatening agriculture in arid and semi-arid areas. Nearly 40% of the world's land surface can be categorized as having a potential salinity problem (Zahran, 2001; Niste et al., 2013). The main cause of salinity is the nutrient imbalance in the soil, which is considered as a constraint influencing the N_2 fixing symbiosis and the survival of both partners (Mohammadi et al., 2012; Niste et al., 2013). Salinity is concentration of dissolved mineral salts comprising cations and anions present in the soil (soil solution) and in water. The principal cations in solution consist of Na^+, Ca^{2+}, Mg^{2+} and K^+ and the major anions are Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-} and NO_3^- (Aggarwal et al., 2012).

The response to saline stress varies among free rhizobia for which the growth is inhibited at 100 mM NaCl, and symbiotic rhizobia, such as *Sinorhizobium meliloti* found to be tolerant to NaCl concentrations ranging from 300 to 700 mM (Zahran, 2001). Some rhizobia isolated from Acacia trees seem to be highly salt tolerant and can grow at a concentration of 500-850 mM NaCl (Zahran, 2001).

Rhizobial strains differ in their ability to tolerate osmotic stress and can use different adaptation mechanisms such as intracellular accumulation of low-molecular-weight organic solutes (Zahran, 1999) including amino acids such as glutamate, N- acetylglutaminyl - glutamine, sugar and polyamines or the accumulation of ions such as K^+. Rhizobia subject to salt stress may undergo morphological alterations, leading to changes in cell morphology and size or modifications in the pattern of extracellular polysaccharides and lipopolysaccharides (Ventorino et al., 2012). These compounds may have an impact on symbiosis because of their implication in the initial steps of the symbiotic interactions. Moreover, some authors have reported that tolerance to salinity may be due to a plasmid-mediated resistance since salt resistance can be rapidly transferred from tolerant to sensitive bacteria, thus extra chromosomal genes can contribute to survival in saline soil (Pereira et al., 2008). Changes in the gene expression appear also to be among the rhizobial adaptation mechanisms to tolerate hyperosmotic stress (Lapez-Go'mez et al., 2013).

Temperature stress

High soil temperature is one of critical factors which can prevent the development of a nitrogen-fixing association between the two symbiotic partners especially in arid and semi-arid regions. The survival of rhizobia in soil is more affected by high temperatures than by low temperatures because it can be deleterious (Niste et al., 2013). In arid regions, high soil temperature affect lives of both free and symbiotic rhizobia (Zahran, 1999). Most *rhizobia* have an optimum growth temperature at 28-31°C and many of them are unable to grow at 38°C (Graham, 1992). However, some rhizobial strains isolated from Acacia have the ability to grow at high temperatures which can reach 44°C (Zahran et al., 1994). Temperature can influence not only the survival of free rhizobia, but also the exchange of molecular signals between the symbiotic partners (Sadowsky, 2005). High temperature can induce an inhibiting effect on bacterial adherence to root hairs, on bacteroïd differentiation, on nodule structure and on legume root nodule's functioning (Zahran, 1999; Alexandre

and Oliveira, 2013). Sudden temperature changes induce synthesis of heat shock proteins (HSP) which can play a protective role and contribute to heat tolerance with no alteration of the internal cell temperature (Yura et al., 2000). Most bacteria have only a small number of HSP but rhizobia seem to present an exception (Alexandre and Oliveira, 2013). The HSP include some proteins such as IbpA and IbpB that show similarity to *Esherichia coli* and other proteins more different in sequence and phylogenetic origin (Alexandre and Oliveira, 2013).

The molecular bases of temperature stress tolerance in rhizobia were studied by comparing the expression of chaperone genes *dnaKJ* and *groESL* in thermotolerant and thermosensitive isolates. These chaperones are characterized by their role as folding modulators, in sequestering and stabilizing a wide range of polypeptides presented in wrong conformational structure (Alexandre and Oliveira, 2013). Nandal et al. (2005) reported that mutants tolerant to high temperature, obtained from a thermosensitive *Rhizobium sp.* strain, exhibited a different protein profile from the wild-type at high temperature and showed overexpressed proteins as well as new proteins. This protein overproduction was confirmed by other studies in mutant strains as DnaK (Alexandre and Oliveira, 2013; Abd-Alla et al., 2014), in chickpea rhizobia as GroEL (Rodrigues et al., 2006) and also in *Mesorhizobium* strains (Laranjo and Oliveira, 2011). *Bradyrhizobium japonicum* shows a total of five *groESL* operons, which only $groESL_{1,4,5}$ are heat inducible and are differently regulated. The $groESL_1$ is σ^{32}-dependent and is highly induced by heat shock. The sigma factor σ^{32} is involved in the control of the heat shock response at the transcriptional level in many bacteria. Unlike GroESL system, DnaKJ system is far less studied but it was characterized in *B. japonicum* and was proved to be under the control of σ^{32} factor (Alexandre and Oliveira, 2013).

The expression of groESL genes from psychrophilic bacteria allowed the increase of *E. coli*'s tolerance to low temperature and decreased the growth temperature's lower limit (Ferrer et al., 2003). Another study reported that thermotolerance was improved by overexpression of native groESL system in *E. coli,* and which may be caused by the folding or refolding activity of the chaperone proteins to misfolded cellular proteins under thermal stress (Kim et al., 2009). The misfolding of intracellular proteins is recognized as a key factor for microorganism's inactivation under thermal stress. The chaperone system like GroEL-GroES has a major role in the defense system; it not only directly interacts with a number of intracellular proteins but also affects some transcriptional networks under stress condition. In fact, this complex forms an enclosed environment for the correct folding of approximately 50% of intracellular proteins under conditions of cellular stress (Kim et al., 2009).

At low temperatures, the cellular membrane rigidity presents a major problem for bacteria, in addition to a decreased rate of enzymatic reactions and the instability of single stranded DNA and RNA (Horn et al., 2007). Some rhizobia strains isolated from wild relative chickpea (*Cicer anatolicum*) collected at high altitude, have demonstrated their ability to nodulate chickpea (*Cicer arietinum*) at low temperatures (9-15°C) (Alexandre and Oliveira, 2013).

Bacterial cold shock response is an immediate and transient response to the temperature downshift and is followed by low temperature adaptation that allows continued growth at low temperatures. Arctic strains of rhizobia respond to cold shocks by synthesizing proteins under their minimal growth temperatures at freezing temperatures as low as -10°C (Cloutier et al., 1992). Proteins induced after cold shocks are designated as cold shock proteins (CSP). These low molecular mass proteins, usually nucleic acid-binding, are well characterized in *E. coli* but poorly studied in rhizobia. A homolog to the *E. coli CspA* gene was detected in *S. meliloti* and reported to be induced by temperature downshift; moreover this *CspA* is known to interact with mRNA, stabilizing the molecule in order to allow translation (O'Connell and Thomashow, 2000).

pH Stress

Either alkaline or acidic agricultural soil has a great influence on the survival or multiplication of rhizobia and can affect both the symbiosis partners. Most leguminous plants require a neutral or slightly acidic soil for growth, especially when they depend on symbiotic N_2 fixation (Zahran, 1999). The optimum pH for rhizobial growth is considered to be between 6.0 and 7.0 (Hungria and Vargas, 2000). In fact, at pH of 5.0-5.5, the nodulation in Acacia trees was absent (Brockwell et al., 2005). The rhizobial strains vary widely in their acidity tolerance. *Rhizobium tropici* and *Mesorhizobium loti* are considered as highly acid tolerant strains (Graham et al., 1994). Some rhizobial strains can withstand and survive even in a very low pH (about 3.5). Alkalinity is less harmful to the survival of rhizobia. Jordan (1984) showed that the majority of these bacteria can tolerate up to pH 9. The same result was found among strains nodulating Acacia (Zerhari et al., 2000) which showed remarkable and sometimes quite extraordinary tolerance to alkaline conditions (Brockwell et al., 2005). For example, rhizobial strains isolated from *A. farnesiana* have shown an ability to adapt and grow at pH 12.0 (Brockwell et al., 2005).

The physiological and biochemical mechanisms of rhizobial adaptation to acidic conditions are various (Graham et al., 1994). These mechanisms include among others the exclusion and expulsion of protons H^+ (El-Hilali, 2006), the increase of potassium and glutamate contents in the cytoplasm of stressed cells (Aaron and Graham, 1991), the change in the lipopolysaccharides

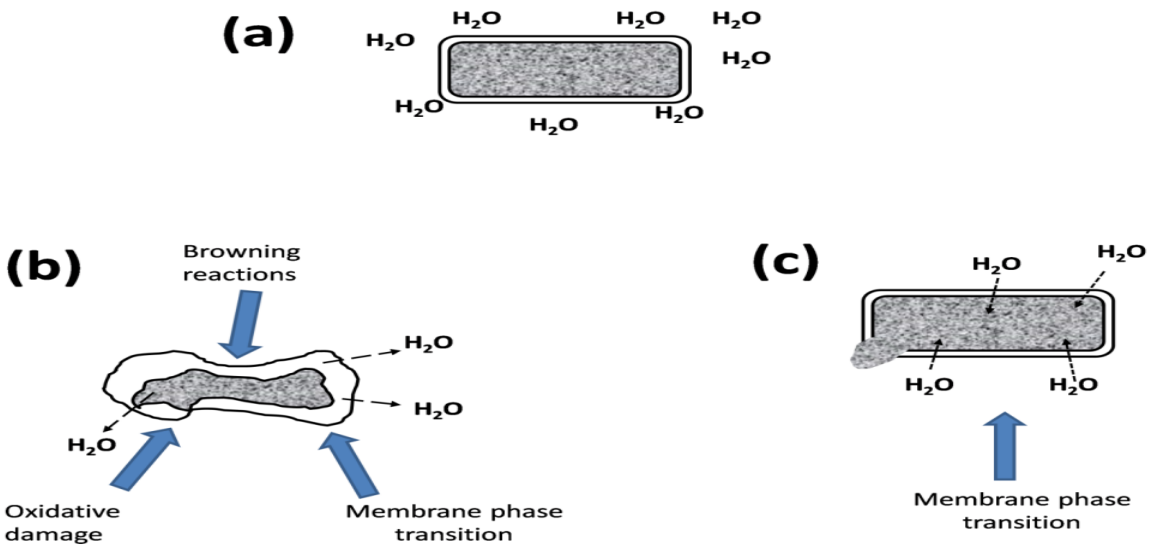

Figure 1. Schematic representation of: (a) hydrated bacterial cell, (b) dehydrated cell exposed to stress damage and (c) dehydrated cell upon rehydration with loss of membrane integrity (Casteriano, 2014).

composition (Vriezen et al., 2007), and the accumulation of polyamines (Fujihara and Yoneyama, 1993). The production of acid shock proteins (ASPs) is another common response contributing to this stress tolerance by conferring acid protection on the bacteria with no alteration of the cellular pH (Foster, 1993). Furthermore, several genes, such as *actA*, *actP*, *exoR*, *lpiA*, *actR*, *actS* and *phrR*, were shown to be essential for rhizobial growth at low pH (Abd-Alla et al., 2014).

However, the negative effect of the alkaline soil's conditions is the unavailability of essential minerals for both rhizobia and host plant such as iron and manganese (Farissi et al., 2014). High pH can also influence the growth of *Rhizobium* and its undergoing nodulation, although some rhizobial species such as *R. leguminosarum bv. trifolii* has been reported to colonize soil at a higher rate and produce nodules at a higher frequency in alkaline conditions (Zahran et al., 1999). Homospermidine, a polyamine present at high concentrations in root nodule bacteria, is also known to accumulate in *B. japonicum* in alkaline conditions, although its function is unknown (Fujihara and Yoneyama, 1993).

Drought stress

Drought stress can present a major agricultural problem which occurs when the available soil water is reduced and the atmospheric conditions induce continuous loss of water by transpiration or high evaporation (Jaleel et al., 2009). The cells under drought conditions are also susceptible to chemical damage as a result of water removal and exposure to the atmosphere (Figure 1). During dehydration, the formation of certain molecules particularly hydroxyl and peroxyl radicals can induce the lipids peroxidation, proteins denaturation and nucleic acid damage (Casteriano, 2014). Reducing sugars may covalently react with the amino side chain of amino acid residues via non-enzymatic browning or Maillard reaction, causing protein damage (Casteriano, 2014).

Drought effects on rhizobial persistence and survival in the soil, on root-hair colonization and on infection by rhizobia can consequently limit the nodulation (Zahran, 1999; Mhadhbi et al., 2011). However, some rhizobial species have shown an ability to tolerate and survive in drought conditions at -3.5 MPa (Abolhasani et al., 2010). The efficiency of these rhizospheric bacteria to persist in severe water deficit conditions can be used to ameliorate drought impact on plants and to help them to tolerate stress by producing physical and chemical changes (Yang et al., 2009). Many species of rhizobia can support severe drought conditions by various adaptive strategies including production of chaperones and sugars, synthesis of stress enzyme 1-aminocyclopropane 1-carboxylic acid, production of exopolysaccharides (Hussain et al., 2014), production of low molecular weight organic compound like trehalose, phosphate solubilization, improved nutrient availability, production of siderophores and phytohormones (Hussain et al., 2014). Under dryness conditions, the aerobic bacteria have shown their ability to use nitrogen oxides as terminal electron acceptors which can help them to survive and grow during periods of anoxia. This may present a great advantage for the survival of rhizobia in soil (Abd-Alla et al., 2014).

Soil fertility

Soil fertility can also affect the biological nitrogen fixation

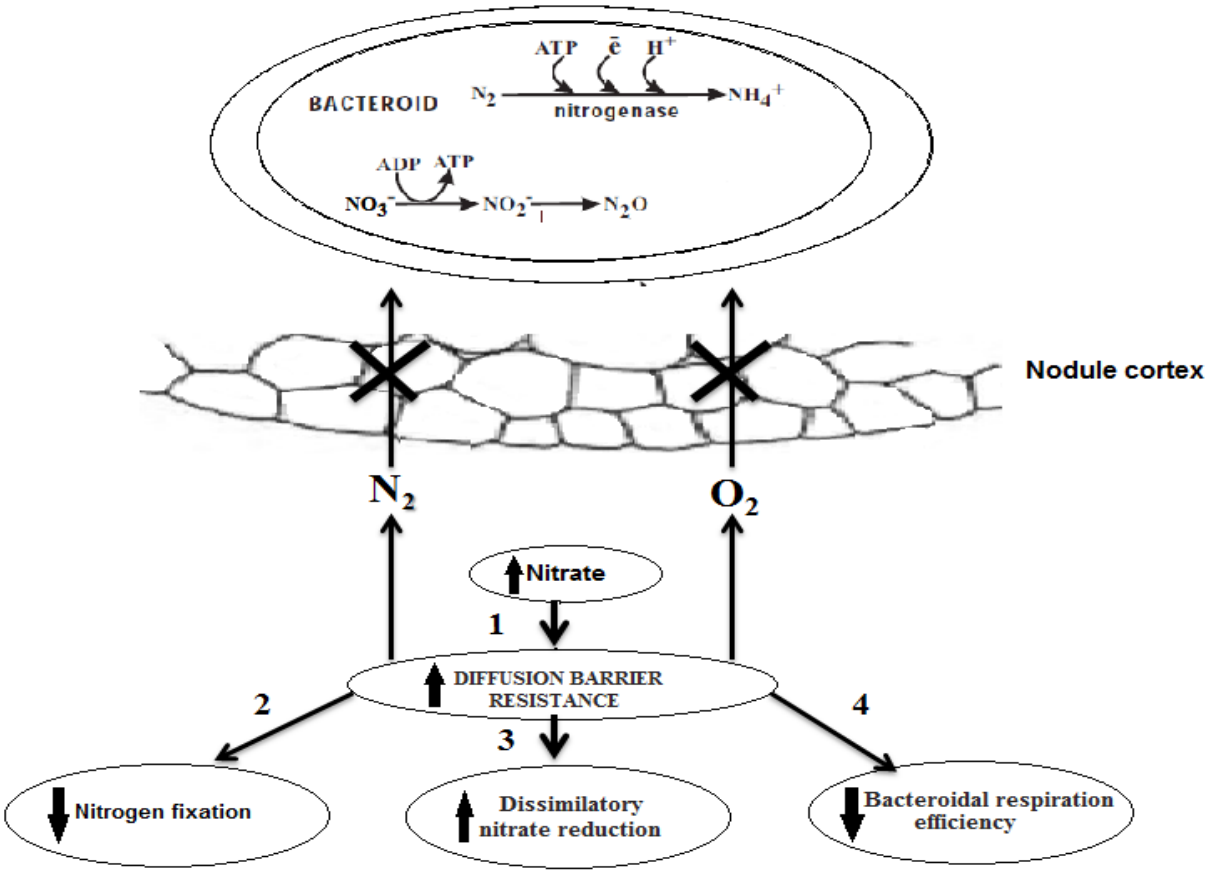

Figure 2. Schematic representation of the effect of the gas diffusion barrier in nodule cortex on bacteroid nitrogen fixation and dissimilatory nitrate reduction (Modified from Luciński et al., 2002). The nitrate availability in the soil induces: 1. Increase of diffusion barrier resistance. 2.4. Decrease of nitrogen fixation and bacteroidal O2 respiration. 3. Lowering of nodule oxygen conditions stimulate simultaneously dissimilatory nitrate reduction.

in *Rhizobium*-legume symbiosis. In fact, an excess of nitrates may cause an inhibitory action on nodulation and N_2 fixation activity (Luciński et al., 2002). The process of this inhibition is not fully understood, although several hypotheses have been proposed (Luciński et al., 2002). Some studies have concluded that legume plantation in soils containing a significant quantity of nitrates can have negative effect on the symbiosis induced by rhizobia (Luciñski et al., 2002) and can inhibit nodulation and nitrogen fixation of acacias (Brockwell et al., 2005). The plant-available N in soil reduced the inoculation response for *A. auriculiformis, A. mangium and A. mearnsii* in pot experiments (Turk et al., 1993). It has been showed in previous studies that the presence of NO_3^- ions reacts negatively on root infection (Wahab et al., 1996), nodule development and nitrogenase activity in legume plants because of the accumulation of nitrite (Luciñski et al., 2002). In the same context, it was demonstrated that the addition of NO_3^- (5-16 mM) to the alfalfa seedlings growth medium reduced significantly the number of rhizobial cells adhering to the alfalfa seedling roots (Zahran et al., 1999). It is also known that the free oxygen concentration inside the nodules is among the major factors that can induce changes in the nitrogenase activity. Oxygen availability in the infected zone nodule is limited, among others, by the gas diffusion resistance in nodule cortex. The presence of nitrate can directly or indirectly influence the effectiveness of resistance to gas diffusion which adversely affects the nodulation and the nitrogen fixation (Luciñski et al., 2002). In the presence of nitrate, both the energy cost of the nitrogen fixation process and the gas diffusion resistance increases, whilst the efficiency of the bacteroid respiration decreases (Figure 2).

Several species of rhizobia can resist to the presence of nitrates during infection and nodulation to a certain degree by induction of hydrogenase expression. This membrane enzyme is characteristic of some diazotrophs and can help some strains to be more tolerant to nitrates (Serrano and Chamber, 1990). Moreover, it was found that hydrogenase contributes to the formation of H^+ gradient

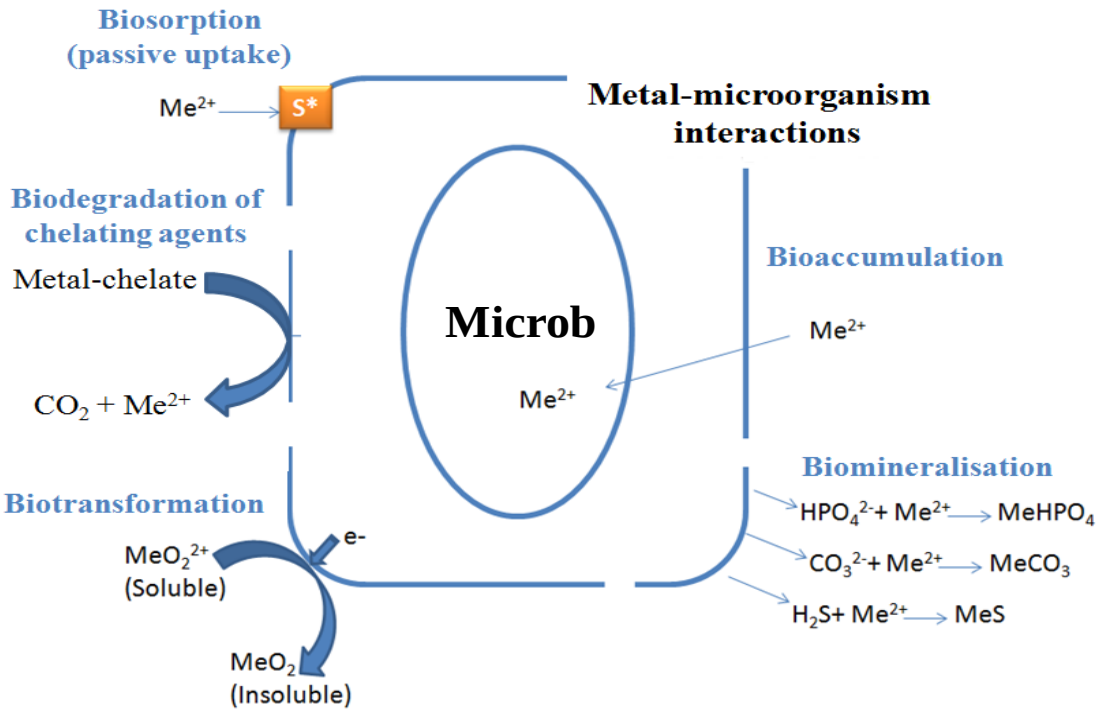

Figure 3. Schematic representation of the metals-microorganism interactions (Modified from Ledin, 2000). Me^{2+}: metal cation. * Functional groups present on the cell wall: carboxyl, phosphodiester, amines, hydroxyls etc.

across bacteroid membrane that enables ATP synthesis (Luciñski et al., 2002).

Heavy metals

Heavy metals are known as the most important inorganic pollutants which persist in the soil over long periods and have ecotoxicological effects on plants and soil micro-organisms. Some metals such as Zn, Cu, Ni and Cr are essential for growth of both rhizobia and their host plants, whereas others such as Cd, Hg and Pb seems to be not beneficial and could be toxic even at relatively low concentrations (Gadd, 1992). When exposed to moderate heavy metal concentrations, soil microorganisms were found to be very sensitive (Giller et al., 1998). Rhizobial response to different types of heavy metals depends on the applied concentrations (El-Hilali, 2006). Hence, cadmium even at considerably low concentration was found toxic for the microsymbiont, inhibited the nitrogenase activity and adversely affected the metabolic activities such as legume's photosynthesis (Ahmad et al., 2012). In contrast, nickel can induce a significant increase in the activity of hydrogenase in bacteroids (El-Hilali, 2006).

Microorganisms have developed resistance mechanisms to support high heavy metals concentrations while ensuring the maintenance of the biological role of essential ions (Figure 3). *Rhizobium* is able to produce huge amounts of extracellular polysaccharide and lipopolysaccharide which sequester most of the extracellular metal and play a role as first-defense barrier against heavy metal stress. However, they were not sufficient to support the highest levels of stress imposed (Mandal and Bhattacharyya, 2012). One of the most common resistance mechanisms is the extrusion of heavy metals from bacterial cell, avoiding accumulation to levels that possibly inhibit growth, or cause cell death (Pajuelo et al., 2011). This mechanism can be complementary to other resistance mechanisms (such as efflux mechanisms) avoiding reentry of expulsed metal, especially in extreme situations. Some of the efflux resistance systems are ATPases and chemiosmotic ion/proton exchangers (Silver and Phung, 2005). In addition, accumulation and complexation of the metal ions inside the cell, biotransformation of toxic metal to less toxic forms, methylation, precipitation and chelation with S-rich ligands like metallotioneins, glutathione, etc. are other metal detoxification mechanisms used by microorganisms (Gusmão et al., 2006). Gram negative bacteria can also synthesize proteins that adhere to the metal and store it in the periplasm in order to keep metals out of the cytoplasm and plasma membrane where the important reactions take place (Pajuelo et al., 2011).

Table 1. Principal mechanisms adopted by rhizobium to tolerate stress factors.

Stress factors	Mechanisms	References
Salinity	- Intracellular accumulation of organic solutes	Zahran (1999)
	- Cell morphology and size changes	Ventorino et al. (2012)
	- LPS and EPS structure changes	Ventorino et al. (2012)
	- Plasmid-mediated resistance	Pereira et al. (2008)
	- Gene expression changes	Lapez-Go'mez et al. (2013)
Temperature	- Synthesis of Heat shock proteins (HSP)	Alexandre and Oliveira (2013), Abd-Alla et al. (2014)
	- Synthesis of cold shock proteins (CSP)	Cloutier et al. (1992), O'Connell and Thomashow (2000)
pH	- Exclusion and expulsion of protons H^+	El-Hilali (2006)
	- Increase of potassium and glutamate level in the cytoplasm of stressed cells	Aron and Graham (1991)
	- LPS structure changes	Vriezen et al. (2007)
	- Accumulation of polyamines	Fujihara and Yoneyama (1993)
	- Production of acid shock proteins (ASP)	Foster (1993)
Drought	- Production of chaperones, sugars, EPS and synthesis of stress enzyme	Hussain et al., (2014)
	- Production of trehalose, siderophores and phytohormones	Hussain et al. (2014)
	- Phosphate solubilization	Hussain et al. (2014)
Nitrate	- Utilization of nitrogen oxides as terminal electron acceptors	Abd-Alla et al. (2014)
	- Induction of hydrogenase expression	Serrano and Chamber (1990)
Heavy metals	- Production of LPS and EPS	Mandal and Bhattacharyya (2012)
	- Extrusion of heavy metals from bacterial cell	Pajuelo et al. (2011)
	- Efflux mechanisms	Silver and Phung (2005)
	- Accumulation of the metal ions inside the cell	Gusmão et al. (2006)
	- Bioreduction of the metals toxicity	Gusmão et al. (2006)
	- Methylation, precipitation and chelation	Gusmão et al. (2006)
	- Synthesis of adhesion proteins	Pajuelo et al. (2011)

These resistance mechanisms are not incompatible and several of them can act simultaneously.

Hence, this review show clearly that even if environmental conditions such as salinity, temperature, acidity/alkalinity, drought, heavy metals, etc. are critical factors affecting different symbiotic steps of the *Rhizobium*-legume association, some microsymbionts strains have developed several mechanisms to tolerate these stress factors and overcome hard environment conditions. Several adaptation mechanisms of rhizobia to persist and to survive under stress conditions have been previously proposed and discussed in other studies and are summarized in Table 1.

CONCLUSION

The environmental conditions play an essential role in the control of legume-*Rhizobium* interactions. They may affect the growth, proliferation, symbiotic process and nitrogen fixation by *Rhizobium* in association with legumenous plants. In this literature review several

symbiotic systems of rhizobia which are tolerant to extreme conditions of salinity, alkalinity, acidity, drought, metal toxicity, fertilizer, etc., were identified.

Under poor conditions, *Rhizobium*-legumes symbiosis is very important because it may be the only way to fix nitrogen; this is why the selection of symbiotic partners tolerant to broad range of unfavorable environmental conditions is essential for agricultural pastoral systems.

Rhizobium-legume response to different environmental stress is complex phenomena that require the intervention of many genetic and biochemical adaptation mechanisms which should be included in future studies. In fact, further knowledge on these mechanisms involved by rhizobia to cope with adverse conditions will allow us to better understand their physiology and to select efficient isolates that can be used in inoculation projects for promoting the plants growth or in engineering genetic.

Conflict of interest

The author(s) have not declared any conflict of interest.

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