Absorbent Food PADS from Meat Packages - Potential Source of Contamination

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The purpose of this study was to quantify the microbial load and to detect potentially pathogenic germs on the surface of absorbent food pads in the packaging of fresh chicken and minced meat marketed in the retail units in Romania. The load in E. coli, as an indicator of faecal contamination, was significantly lower than that of Enterobacteriacea and coliform germs. Congo red-binding E. coli strains have been identified. The affinity for the Congo red dye is considered a phenotypic marker for the presence of curli fibers. These surface formations give the bacterial strains the ability to form biofilm on various surfaces. Their presence also correlates with the property of these bacterial strains to trigger severe infections in humans. These strains showed 100% resistance to fourth-generation cephalosporins, 87.5% to third-generation cephalosporins and 75% to quinolones. A single strain was resistant to meropenem. All the tested strains were susceptible to gentamicin. Coagulase-positive staphylococci were identified in 40% of the analyzed samples. Of the 30 strains of Staphylococcus spp. tested (coagulase-positive and negative), 63.3% proved to be methicillin-resistant and 43.3% had resistance to sulfamethoxazole-trimethoprim. In one sample, it was detected Salmonella infantis. Thus, it can be appreciated that these absorbent food pads may be a source of direct contamination with potentially pathogenic and / or antibiotic-resistant germs for the persons who manipulates them in their domestic environment, as well as a source of cross-contamination of food and other surfaces in the kitchen.

Keywords: absorbent food pads, animal food stuff packaging, microbiological examination, in-home/ domestic contamination

In recent years, the consumers growing demands on food safety and quality, as well as on purchasing facilities, made him give up on buying from butcheries, in which the butcher handles and divides parts of the carcass and meat products as directed by the customer. Instead, the consumer focused on the refrigerated display cases of large stores, where the same products are pre-packaged, weighed and labeled. Also, strict health barriers imposed by international trade in order to reduce consumer health risk, forced meet producers, processors and distributors of animal food stuff (and not only), to adjust their technology and comply with the imposed requirements in order to remain on the market.

Thus, meat products are often found in polystyrene trays or plastic containers, covered with a protective foil and packaged in a protected atmosphere so as to maintain their freshness for a period of time. However, the meat contains its own fluids which, over time, will leak and accumulate in the container, what most consumers find disagreeable [21]. For this reason, absorbent materials are often placed in these containers. However, regardless of how the meat juice is found in the container, free or absorbed, it constitutes both an environment conducive to development of psychrophilic altering bacteria and a survival and propagation environment for pathogenic microorganisms that can cause food borne diseases [19, 21]. For example, *Salmonella* can adapt, survive and even multiply at *p*H values of 3.99 to 9.5, at a concentration of up to 4% NaCl and at temperatures of 2°C to 54°C [35].

The magnitude of diseases caused by pathogenic microorganisms in food, globally and in developing and underdeveloped countries, in particular [40], but also the concern of highly developed countries to avoid food waste [37], has attracted the attention of European legislators and researchers on the need to act in increasing product lifetime and reduce consumer risk through *active* and *smart* food packaging [23, 31]. In accordance with existing Community legislation on products that get in contact with foods, *active materials and objects* are defined as those materials and objects which consist of *active* elements that can be released into packaged food or serving to absorb the substances coming from them in order to extend their shelf life, to maintain or improve their quality [49, 50].

Numerous antimicrobial chemicals [16, 26, 33], complex chemical mixtures [21], nanoparticles [15], essential oils [29, 34] or bacteriophages activity [19] have been tested over time to be incorporated into such active materials in order to limit the development of pathogenic and altering microorganisms in packaged foods.

Even though these substances have proven to be effective in the studies, the agreement on their widespread use is subjected to legislation and EFSA's (European Food Safety Authority) approval. The main criteria to be fulfilled are that they should not pose risks to consumers by themselves and should not mislead the consumer by masking the incipient altering phases of the food. Also, the product label should inform the consumer that such active materials are contained in the packaging [49, 50]. On the other hand, the limitation in the use of these substances is also due to the current tendency of consumers to choose the most natural foods without additives, preservatives etc.

In this context, the purpose of the present study was to quantify the microbial load and to detect potentially pathogenic bacteria for humans on the surface of the absorbent food pads in the packaging of fresh chicken meat and minced meat marketed in retail units in Romania, to see if there is a risk of domestic / in home contamination. This risk arises from the need of handling these materials,

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bearing in mind the obligation of citizens to selectively collect household waste [54, 55]. Concretely, in this situation, the consumer should place the packaging at the recycling bin and the absorbent material, as it is soaked with meat fluids, at the bin for biodegradable waste collection.

Experimental part

Materials and methods

There were analyzed 24 samples, consisting of absorbent food pads (AP) from packages with fresh chicken meat, with and without skin, and minced beef and chicken, purchased from a retail unit from the city of Timisoara. Products have been selected from several producers, in their shelf-life period, with the expiration date as far as possible from the time of purchasing.

Absorbent materials were taken sterilely immediately after opening the package and immersed in bottles containing 50 mL of peptone water, thus constituting the initial suspension. The vials were put in a stomacher for 5 minutes. Samples were processed immediately.

In 15 samples (six AP's from packages with products with skin, six AP's from packages with skinless products, and three AP's from packages with minced meat, two with beef and one with chicken), the quantification of germs from the *Enterobacteriaceae* group, of coliforms and *Escherichia coli* germs, as well as the identification of coagulase-positive staphylococci and *Salmonella* spp. was performed. Samples were analyzed in accordance with SR EN ISO 21528-2/2017 (*Enterobacteriaceae*) [44], SR EN ISO 4832/2009 (coliforms) [45], SR EN ISO 16649-2/2007 (*Escherichia coli*) [46], SR EN ISO 6888-1/2002/A1/2005 (Coagulase-positive staphylococci) [47], SR EN ISO 6579-1/2017 (*Salmonella* spp.) [48]. For the detection of *Salmonella* spp. in the analyzed samples, the biochemical confirmation was done by inoculating on the TSI (triple-sugar-iron) agar and MIU agar (motility-indole urease). Positive samples were subjected to seroagglutination (according to the Kauffmann-White scheme) by using a *Salmonella* O polyvalent antiserum (A-G), and the serovar identification was performed by API 20E method.

For a most accurate rendering of the microbial load relative to the surface unit, expression of the results was done in log CFU/cm^2 [10]. The average surface area of the analyzed materials was 96 cm^2.

A batch of 22 samples (including the above 15, except for the beef meat) were analyzed for the phenotypic detection of *E. coli* strains belonging to the ExPEC pathotype, based on their ability to express adhesion factors of the curli fiber type [28], proteic formations that have chemical affinity for the Congo red dye [20]. This property was tested by inoculating selected strains on TSA medium (Trypticase Soy Agar) with the addition of bile salts (1.5 g/L) and Congo red dye (0.3 g/L) [**7**]. After incubation at 37°C for 24 hours, the colonies that had the center red and were surrounded by a lighter halo as a result of binding the dye, were considered positive. The plates with confluent colonies and those where colonies did not show a halo, were excluded or considered negative.

In all 24 samples, the identification of biofilm-forming staphylococci was performed by their ability to form black colonies on a culture medium containing Congo red dye. The medium was prepared by modifying two recipes, the first one for the evidencing of coagulase-negative biofilm-forming staphylococci [18], and a second one used to evidence the same property in MRSA (methicillin-resistant *Staphylococcus aureus*) [24]. Thus, glucose (8 g/L) and Congo red dye (0.8 g/L) were added to the BHI (Brain Heart Infusion Agar, Oxoid 47 g/L) medium. The plates were incubated at 37°C for 24 h and then maintained for another 24-48 h at room temperature. The occurance of black colonies was expected.

Antibiotic susceptibility testing was performed for part of the isolated *E. coli* and staphylococci strains, by disk diffusion method [11] and the interpretation of the inhibition zone diameter was performed according to the EUCAST provisions [12].

For the statistical interpretation of the values, there have been used the ANOVA single factor and the t-Test: Two-Sample Assuming Equal Variances. According to this tests, if the result of comparisons is $p<0.05$ (significant differences) and it is obtained on a small specimen, the result can be considered important, unlike the situation in which the result of comparisons is $p>0.05$ (the differences are not significant) on a small specimen, when the result is considered to be inconclusive.

Results and discussions

Table 1 shows the values of microbiological indicators (*Enterobacteriaceae*, coliforms and *E. coli*) for each sample.

In table 2 are rendered the mean values of microbial loads and the prevalence of coagulase-positive staphylococci on the surface of AP collected from the packages containing chicken meat with skin and skinless, and those containing minced chicken and beef.

After comparing the values of the microbiological indicators (*Enterobacteriaceae*, coliforms, *E. coli*) using ANOVA single factor, for each sample batch, the differences were found to be significant: $p<0.05$ for skin chicken AP, $p<0.05$ for skinless chicken AP, and $p<0.05$ for minced beef AP. Following the t-Test, in all three batches, the *E. coli* load was significantly lower than that of *Enterobacteriaceae* ($p<0.05$) and coliforms ($p<0.05$).

Sample type	Identification no.	log CFU/cm^2		
		Enterobacteriaceae	Coliforms	*E. coli*
Chicken fresh meat with skin	1	6.67	6.13	1.92
	2	5.37	4.94	2.48
	3	5.70	5.69	2.49
	4	5.36	4.99	2.41
	5	3.44	2.99	0.99
	6	3.92	3.14	2.07
Skinless chicken fresh meat	7	1.67	1.38	1.24
	8	2.41	2.11	1.56
	9	5.36	5.07	1.79
	10	5.67	5.63	1.86
	11	3.45	3.24	1.47
	12	3.99	3.69	0.79
Minced meat	13. beef	3.17	2.64	<0.31
	14. beef	3.33	2.55	<0.31
	15. chicken	5.29	4.22	1.16

Table 1
ENTEROBACTERIACEAE, COLIFORM BACTERIA AND *E. COLI* VALUES FOR EACH SAMPLE AND PREVALENCE OF COAGULASE POSITIVE STAPHYLOCOCCI ON APs SURFACES

Table 2

AVERAGE MICROBIAL LOAD (*ENTEROBACTERIACEAE*, COLIFORM BACTERIA, *E. COLI*) AND PREVALENCE OF COAGULASE POSITIVE STAPHYLOCOCCI ON APs SURFACES

Sample type (AP)		No. of samples (sample identification number)		*Enterobacteriaceae*		Coliform bacteria		*E. coli*		Coagulase-positive staphylococci			
				log CFU/cm²						Positive samples		%	
Chicken (with skin)		6 (1,2,3,4,5,6)		5.97		5.54		2.26		2		33.3	
Chicken (skinless)		6 (7,8,9,10,11,12)		5.07		4.96		1.57		2		33.3	
Minced meat	beef	2	3	3.34	4.83	2.60	3.77	<0.31	0.8	1	2		66.6
	chicken	1	(13,14,15)	5.29		4.22		1.16		1			
Total		15								6		40	

After comparing the values obtained for *Enterobacteriaceae*, there were no significant differences ($p>0.05$) between batches (with skin, skinless, minced meat), as well as for coliforms ($p>0.05$). However, significant differences between batches were found in *E. coli* ($p<0.05$) (ANOVA single factor), due to the very low bacterial load on minced meat AP compared to skinless chicken parts AP ($p<0.05$) and chicken parts with skin AP ($p<0.05$) (t-Test).

Bacteria from the *Enterobacteriaceae* family, coliform germs and *E. coli* are the most commonly used hygienic indicators in the food industry, both for food and for surfaces that come in contact with them, due to simple and rapid methods by which they can be identified. Moreover, in the same sample, the presence of *E. coli* has dual significance, both an indicator of faecal contamination and an index organism for the potential presence of enteric pathogens such as *Salmonella* [5].

With regard to these absorbent materials, as a component part of food packaging, European legislation makes no reference to microbiological criteria [49, 50]. In contrast, in national legislation, there are certain provisions regarding aerobic mesophilic count (1 cfu/mL capacity, up to 2 cfu/mL capacity if coliform bacteria are absent) and the need for coliform bacteria to be absent. For packaging, hygienic indicators such as *Enterobacteriaceae*, coagulase-positive and haemolytic staphylococci, and molds are not regulated [53]. Also, the difficulty of comparing the data obtained in this study with the requirements of the legislation is also due to the fact that the reporting of the results is done per unit of volume, whereas the samples analyzed in this study were pieces of absorbent material with a certain surface area which, after removal of the contained product, majority proved to be dry, not expressing retained fluids from the meat.

The fact that the coliforms load did not vary significantly from that of *Enterobacteriaceae*, after comparing the values of the microbiological indicators, for each batch, may be due to the presence of psychrotrophic strains of coliforms belonging to *Citrobacter, Enterobacter, Escherichia, Klebsiella* genders, which have the ability to multiply at refrigeration temperature [5]. Also, the fact that the values of *Enterobacteriaceae* and coliforms did not vary significantly, irrespective of the packaged product (with skin, skinless and minced meat), even if the mean value was higher for the batch with skin than the skinless one and the latter to the minced meat batch (table 1), may be due to the presence of the same strains. Even if the initial degree of contamination is lower, the existence of the appropriate substrate (exposure of a larger free surface through lack of skin and mincing) allows therefore the intense multiplication of these microorganisms [4].

The low *E. coli* load can be due to the fact that it is generally a germ of faecal origin, and it's multiplication at low temperatures is limited [4], but also to the identification method used, which only allows the growth of the strains that develop at 44°C [46]. The fact that it is used as both hygienic and faecal contamination indicator for minced meat [51] can explain the reduced load on the surface of the absorbent materials, in general, and on those from the minced meat packages, in particular.

However, regardless of the contamination origin of these materials, which has not been the objective of this study, the contamination level is considered high. The risk for the consumers who will handle these materials consists in:

-the fact that all pathogenic *E. coli* strains and non-typhoid *Salmonella* serovars belong to the *Enterobacteriaceae* family,

-the existence of opportunistic and nosocomial pathogens within the coliform group (*Klebsiella, Citrobacter*) and within the *Enterobacteriaceae* family (*Providencia, Serratia*),

-the possibility of dissemination, through these materials, of extended-spectrum-beta-lactamse producing bacteria and other multidrug resistant germs belonging to *Enterobacteriaceae* family [5].

The prevalence of coagulase-positive staphylococci was 40%. The prevalence for both chicken with skin and skinless

Table 3

ANTIBIOTIC SENSITIVITY PATTERNS FOR STAPHYLOCOCCAL AND *E. COLI* ISOLATES

Isolates	Antibiotics	Disc content	Resistant strains		Intermediate strains		Susceptible strains	
		µg/disc	No.	%	No.	%	No.	%
E. coli 8 strains	Ciprofloxacin	5	6	75			2	25
	Ofloxacin	5	6	75	1	12.5	1	12.5
	Cefixime	5	7	87.5			1	12.5
	Cefepime	30	8	100				
	Gentamicin	30					8	100
	Meropenem	10	1	12.5	1	12.5		
Staphylococcus spp. 30 strains	Methicillin	5	30	100				
	Oxacillin	5	3	10			27	90
	Cefoxitin	30	19	63.33			11	36.66
	Sulfamethoxazole/ Trimethoprim	25	13	43.33	3	10	14	46.66

chicken APs was 33.3% and for minced meat APs, 66.6% (table 2). Antibiotic resistance was tested for 30 of the isolates, both coagulase-positive and coagulase-negative, and the results are shown in table 3.

Both coagulase-positive and coagulase-negative staphylococci may cause food-borne illnesses [30]. Moreover, these germs are believed to possess genes conferring multiple antibiotic resistance [32].

The strains classification as methicillin-resistant (MRS) was based on cefoxitin susceptibility testing, with 63.3% of the evaluated strains being resistant. Cefoxitin is considered a highly sensitive and specific marker for resistance mediated by the expression of mec A/mec C genes, including heterogeneous resistance strains. The disc-diffusimetric method for oxacillin susceptibility testing is discouraged, and the interpretation of inhibition diameter zone is no longer included in the EUCAST tables because of the poor correlation with the presence of the mec A gene [12, 13]. As a result, only 10% of the tested strains showed resistance to oxacillin.

Testing susceptibility to trimethoprim/sulfamethoxazole (SXT) is justified on the one hand by its inclusion in the highly important antimicrobials group for human use [43], being the first therapeutic option for uncomplicated urinary infections and skin and soft tissue infections, and on the other hand, due to the increasingly frequent reporting of SXT resistance of methicillin-resistant *S. aureus* (MRSA) strains isolated from clinical cases, globally. Resistance prevalence ranges from 0% in North America to 100% in South America, with Europe reporting a low to medium resistance: 1% in Spain, 14.5% in Italy, and 67% in Portugal [8]. In the present study, 43.3% of the tested strains showed resistance to SXT.

Congo red binding *E. coli* strains were identified in 8 samples out of 22 tested (fig. 1). Thus, on the surface of skinless chicken parts APs, the prevalence was 46% (6 positive samples out of 13 tested) and on the chicken parts with skin APs, 25% (2 positive samples out of 8 tested). No such strains have been identified on the single minced chicken meat AP tested. For the eight positive strains, the antibiogram was performed, and the results are shown in table 3.

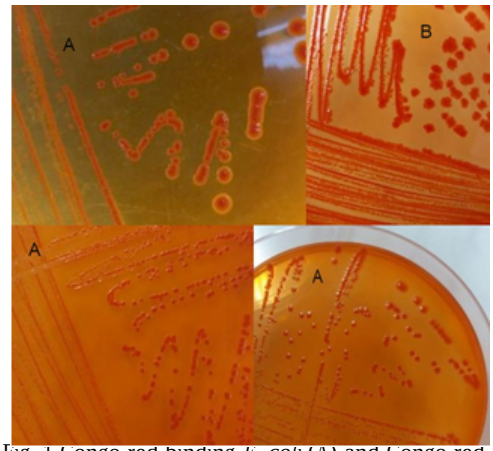

Fig. 1 Congo red binding *E. coli* (A) and Congo red non-binding *E. coli* (B)

Higher prevalence on the surface of the absorbent material inside the skinless chicken parts containers is explained by the fact that APEC strains induce septicemic infections in birds.

The ExPEC (extra-intestinal pathogenic *E. coli*) pathotype includes the following subgroups: UPEC (uropathogenic *E. coli*), NMEC (neonatal meningitis associated *E. coli*), SePEC (sepsis associated *E. coli*) and APEC (avian pathogenic *E. coli*). APEC strains are at the origin of avian colisepticemia, a disease that produces large economic losses in poultry flocks [2].

Genomic studies have shown that certain APEC strains are very similar to those belonging to the UPEC and NMEC subgroups [22, 39]. Thus, the hypothesis of the zoonotic potential of the avian strains has been outlined [9] and, moreover, the possibility of transmitting these strains to humans through chicken meat; the illnesses they produce are called food-borne urinary tract infections (FUTI) [27].

It is known that most Gram-negative pathogens exhibit fimbria, which are extracellular bacterial formations that facilitate their adhesion to the surface of the various epithelia of the host. Within the ExPEC pathotype, such adhesion factors, common to the UPEC and APEC subgroups, are curli fibers too [1]. The presence of these surface formations in ExPEC strains is strongly correlated with their ability to form biofilm on various surfaces as well as triggering severe human infections [3].

Since curli fibers have chemical affinity for Congo red dye [20], it has been used in studies as a phenotypic marker for both the identification of *E. coli* strains having the ability to express these fibers as a pathogenicity factor [28], and for the discrimination of APEC strains from non-pathogenic strains [7].

Antibiotics tested for Congo red binding *E. coli* strains belong to the group of critically important antimicrobials for human use, with the highest (cefixime, cefepime and quinolone) and high priority (gentamicin, meropenem), that are used in urinary infections and not only [43]. Resistance to fourth generation cephalosporins was foud to be 100%, and to third generation cephalosporins, 87.5%. Resistance to quinolones (ciprofloxacin and ofloxacin) was 75%. A single strain showed resistance to meropenem, and resistance to gentamicin was not reported.

There have not been identified biofilm-forming staphylococci, by the applied phenotypic method. The color of the colonies ranged from pink to dark red and brown, without any black appearance (fig. 2).

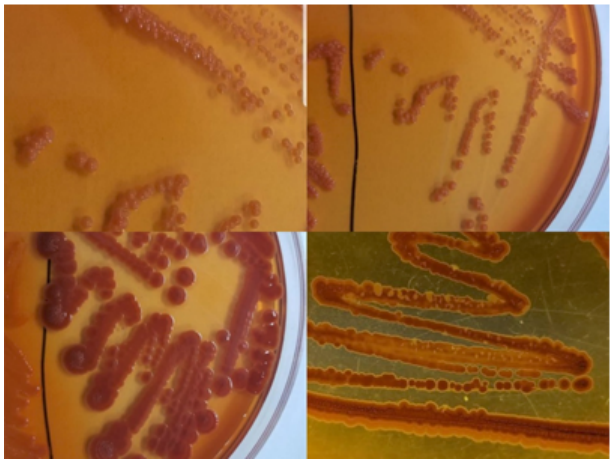

Fig. 2 The appearance of staphylococcal colonies, ranging from pink to dark red and brown colour, without any black appearance

Salmonella has been identified on the surface of a single AP that belongs to a container with fresh chicken meat with skin. Following serotyping, *Salmonella enterica subsp. enterica* serovar *infantis* was identified.

Even if European legislation provides as a safety criterion the testing of fresh poultry meat for the presence of *Salmonella typhimurium* or *Salmonella enteritidis* [52], the *S. infantis* serovar was reported to be the fourth serovar that caused food borne diseases in humans, in Europe, during 2016. Also, along with *Salmonella kentucky*, it was the most commonly reported serovar showing multidrug resistance [14]. Both nationally and in Europe, it has been

the most frequently identified serovar in chicken meat in recent years [14, 25, 38].

These absorbent food pads can become contaminated from a vary of sources, like the packaging itself, the packed meat or by handling personnel along the processing and packaging stages, but the aim of this study was not to identify these sources, but to assess the risk which these materials present, as a source of in-home contamination.

Over the past 40 years, many studies have aimed to assess the degree of microbial contamination of different kitchen surfaces and objects and if the identified pathogens can be the cause of food-borne diseases, as well as to find a correlation between respecting or not some properly in-home hygiene practices and the onset of these morbid entities [6, 36, 17, 41]. Moreover, Romania reported that in 1999-2000, 94% of food-borne illnesses occurred in the domestic environment [42].

These absorbent materials, as a component part of the packaging, have never been evaluated separately, in this context, rather giving importance to the meat, as source of contamination for humans and for different surfaces in the kitchen. However, with regard to products intended to be consumed after heat treatment, as is the case with those analyzed in the present study, the manufacturer warns the consumer, by labeling, about the need of subjecting the product to heat treatment and, moreover, about the need to clean the surfaces with which the raw product came into contact.

The bacteriological risk that these absorbent materials pose to the consumer by manipulation in the domestic environment is enforced by the stringent necessity of selective collection of household waste in Romania, an acute problem which attracts financial sanctions by not achieving the objectives imposed by the European Union. National and local authorities have already implemented selective waste collection systems, both in urban and rural areas, along with providingin citizens with information guides, in line with current legislation. According to these guidelines, recyclable materials, when separately collected in a different bin, should not contain traces of organic matter. Thus, the plastic containers in which the meat products are packaged must be collected separately from the absorbent material inside, which will be assimilated to non-recyclable waste [55]. Thereby, handling this absorbent food pads becomes mandatory.

Conclusions

The present study highlights that the absorbent food pads from the packaging of different categories of fresh chicken and minced meat can be a source of potentially pathogenic germs for humans (*Salmonella infantis*, coagulase-positive staphylococci, *E. coli*), as well as of methicillin-resistant staphylococci and *E. coli* strains with high prevalence of resistance to critically and highly important antimicrobials for human use. The selective collection of household waste in the domestic environment requires the consumer to handle these absorbent materials, thereby creating a risk of spreading these germs on kitchen surfaces and on hand wiping materials and a risk for direct contamination.

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