

**Original article:****Diarrhea Infections in North-Eastern Ukraine: Evolution of Epidemic Process**Nina Malysh<sup>1</sup>, Mykola Chemych<sup>2</sup>, Viktoriya Zadorozhna<sup>3</sup>, Alla Podavalenko<sup>4</sup>, Svitlana Birukova<sup>5</sup>**Abstract:**

**Background:** The significance of some infection sources, pathways of pathogen transmission and etiological structure of diarrhea infections (DI) have changed. Many issues of DI epidemiology are still insufficiently studied, which prevents the organisation of quality epidemiological surveillance and diseases control. **Objectives:** To study the DI epidemic situation (ES) in North-Eastern Ukraine and to identify the factors that determines the characteristics of epidemic process (EP). **Material and Methods:** The reports of Ministry of Health of Ukraine (1960-2018) and Head Statistics Administration of Sumy Oblast (2001-2018) have been used. The biological properties of 40 strains of *K. pneumoniae*, 50 - *S. aureus*, 40 - *E. cloacae* recovered from the feces of patients with DI have been studied. **Results:** It has been found that the EP of DI is characterized by a sharp decrease in the incidence of typhoid fever and shigellosis, increase in the incidence of salmonellosis and dominance of DI caused by other specified or unspecified pathogens (SDI and UDI) in the nosological structure ( $p < 0.05$ ). The incidence of DI caused by *Klebsiella*, *Staphylococcus*, *Enterobacters* exceeds the incidence of salmonellosis, shigellosis, typhoid fever ( $p < 0.05$ ). Opportunistic pathogens (OP), which are pathogens of DI have antilysozyme activity and adhesion ability, which proves their pathogenic potential. The demographics indirectly affect the incidence of DI. Access to water supply, sewerage, hot water supply is correlates with the incidence of DI. **Conclusion:** The system of epidemiological surveillance over DI in Ukraine needs improvement by optimisation its information and analytical subsystems aimed at objective epidemiological diagnostics and ES forecasting.

**Keywords:** DI; OP; correlation.

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**Introduction:**

Diarrhea infections (DI) are among the most widely spread infectious diseases in the world. World Health Organisation reports 1.7 milliard cases of DI registered annually in the world<sup>1</sup>. DI incidence in both developing and economically developed countries is quite high<sup>2, 3, 4, 5</sup>.

DI epidemiology, influenced by globalization, migration increase, international tourism on the background of general environmental degradation, decrease of immunological status of the population, strengthening of the pathogenic potential of causative agents has changed. The significance of some infection sources, pathways of pathogen transmission

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and etiological structure of DI have changed<sup>6,7,8</sup>.

Many issues of DI epidemiology are still insufficiently studied, which prevents the organisation of quality epidemiological surveillance and diseases control.

#### Objectives:

To study the DI epidemic situation (ES) in North-Eastern Ukraine and to identify the factors that determines the characteristics of epidemic process (EP).

#### Materials and research methods:

The reports of Ministry of Health of Ukraine (1960-2018) and Head Statistics Administration of Sumy Oblast (2001-2018) have been used. The biological properties of 40 strains of *K. pneumoniae*, 50 - *S. aureus*, 40 - *E. cloacae* recovered from the feces of patients with DI have been studied. Antilysozyme activity (ALA) of opportunistic pathogens (OP) was determined using cup-plate technique according to the method of O.V. Bukharin et al. in the range of lysozyme concentration from 5 to 25 µg/ml (the strain *Micrococcus lysodecticus*(ATCC 10240) was used as a test culture). The strains were considered highly active at lysozyme neutralization if concentration was greater than 25 µg/ml, intermediate level-10-20 µg/ml, lowlevel - 5 µg/ml. The maximum value of lysozyme concentration in the medium at which the growth of the indicator strain was still observed was taken as ALA level of the studied cultures<sup>9</sup>. The adhesive properties of OP were determined using the method of V.I. Brilis et al. Adhesion was considered to be zero at average adhesion index from 0 to 1.0, low – at mean value of adhesion from 1.01 to 2.0, average – from 2.01 to 4.0, high – more than 4.0<sup>10</sup>.

The epidemiological, microbiological and statistical methods of research have been applied. Statistical processing of the study results have been carried out using computer programmes Microsoft Office Excel 2010, SPSS, 12, Statistica 6.

**Ethical clearance:** Not required

#### Results and discussion:

It has been established that during 1960-2018 there have been significant changes in EP of DI. The ES was characterized by the decrease in the incidence of typical intestinal anthroponoses (typhoid fever and shigellosis). Typhoid fever incidence decreased from 7.4 per 100 000 people in 1960 to 0.3 - in 2002. Since 2003, the cases of typhoid fever in North-Eastern Ukraine have not been registered (Fig. 1). Shigellosis EP in the period of 1960-1975 was marked by the gradual incidence increase from 100.7 per 100 000 people up to 625 per 100 000 people. Starting 1976 there was a decrease to 0.6 per 100 000 people in

2018 on the background of insignificant rises in shigellosis incidence, (Fig. 2).

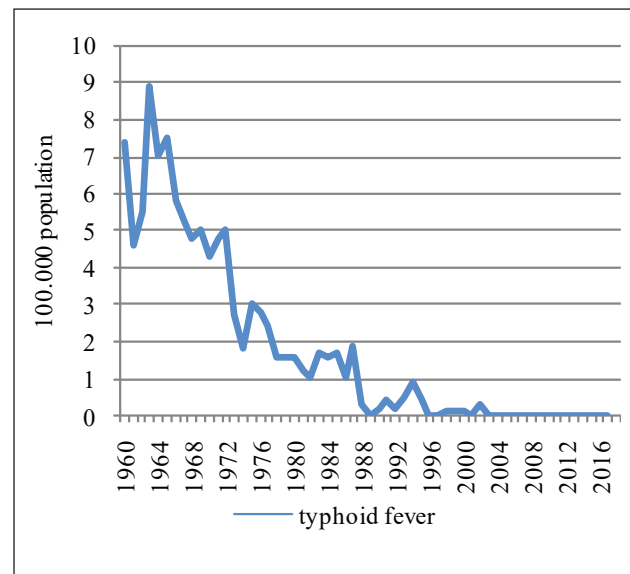


Figure 1. Incidence of typhoid fever

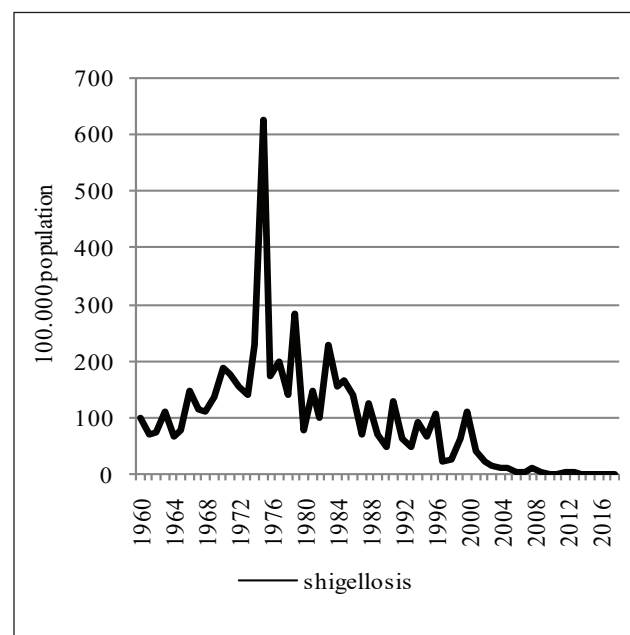


Figure 2. Incidence of shigellosis

It is well known that the main risk factors for the rapid spread of DI are poor sanitation and hygiene, low cultural practices of population. The main causes of the cases were microbially contaminated products of dairy industry, public catering establishments, drinking water, as well as poor sanitary and technical state of the kitchen facilities of preschool institutions, gross violations of food technology.

The Ministry of Health of Ukraine issued a decree regulating the main principles of sanitary and epidemiological surveillance and anti-epidemic

measures in respect of DI in order to improve DI prevention during tense ES, when high incidence of shigellosis, outbreaks of DI were registered<sup>11</sup>.

Taking into account the above, it can be assumed that many factors contributed to the decrease in the incidence of intestinal infections (typhoid fever and shigellosis), where a person is the only source of pathogens, and one of them is greater control over compliance with sanitary and hygienic rules, improvement of social and economic living conditions in Ukraine in general and Sumy Oblast in particular. Timely detection of DI pathogens carriers during preventive and periodic medical examinations of employees of food production facilities, continuous state sanitary and epidemiological supervision of compliance with sanitary legislation during procession, transportation, storage and sale of food and food stock; water supply facilities, certainly affected the improvement of ES. Automation of production processes in the food industry and public catering establishments, use of modern equipment allowed excluding people from the technological process, reducing the risk of microbial contamination of food.

At the same time, it should be noted that the risk of ES worsening constantly exists taking into account the epidemiology of the abovementioned diseases. Thus, 3-5 cases of typhoid fever are detected annually in foreign citizens or tourists returning to Ukraine from endemic area of typhoid fever. The median incidence of shigellosis in 2011-2018 in the neighboring Kharkiv Oblast is 11.6 times higher and is 18.6 per 100.000 people.

On the background of reduced incidence of intestinal anthroponoses, salmonellosis EP was characterized by the incidence increase from 3.6 per 100.000 people in 1960 to 19.3 in 2018 (Fig. 3). The incidence rate of other DI or caused by other specified or unspecified pathogens (SDI and UDI) was high, despite the decrease from 499.4 per 100.000 in 1960 to 132.1 in 2018 (3.8 times) (Fig. 4).

Consequently, the implemented sanitary and hygienic measures were not effective enough in respect of salmonellosis. During 1960-2018 the incidence increased by 5.5 times. The abovementioned points to the ineffectiveness of unified preventive measures and proves the EP autonomy of various nosological entities of DI in general, and salmonellosis in particular. The wide application of centralized fattening of animals in livestock farms, the use of industrial methods of meat and meat food production contributed to *Salmonella* dissemination in animals

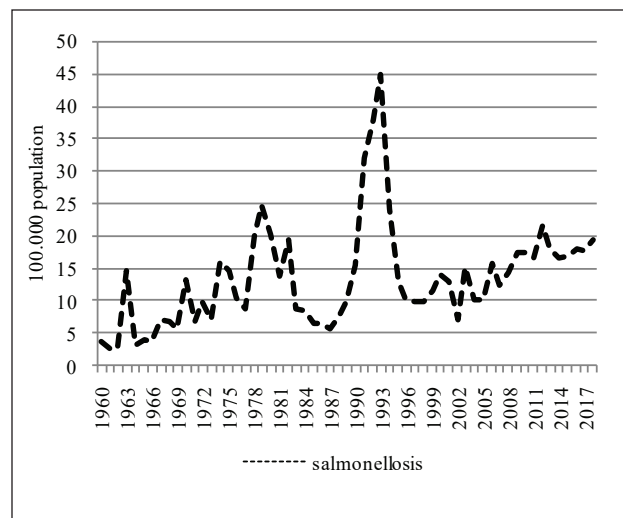


Figure 3. Incidence of salmonellosis

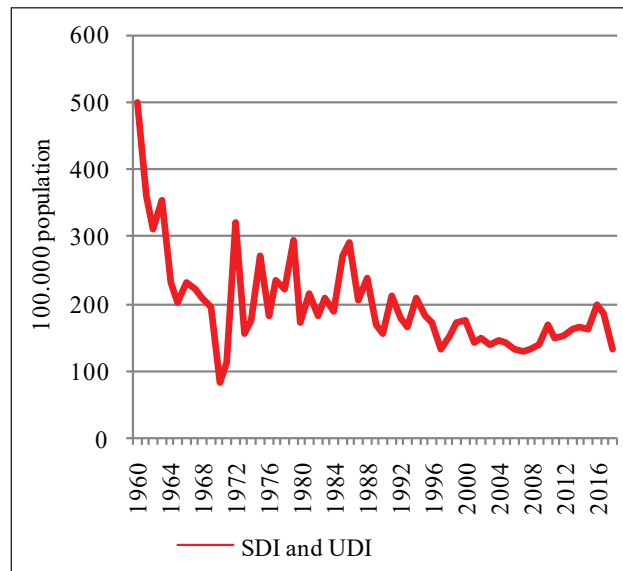


Figure 4. SDI and UDI incidence

and environment, which affected the dynamics of salmonellosis incidence in people. The incidence rate of the disease is mainly due to non-compliance with hygiene and sanitary regulations, violations of food technology. Taking into account the unfavorable salmonellosis situation, the Ministry of Health of Ukraine issued salmonellosis prophylaxis decree in 1985<sup>12</sup>. The measures were mainly aimed at preventing contact transmission and transmission via fomites.

The genus *Salmonella* includes more than 2000 serovars. Serovars, most often causing the development of salmonellosis (*S. Enteritidis*, *S. Typhimurium*, *S. Infantis*) in humans and animals do not cause clinical manifestations of the disease or infection develops subclinically. As salmonellosis ES did not improve and, moreover, this disease

became an issue for animals, consequently, the Ministry of Agrarian Policy and Food issued a decree in 2016 with instructions for prevention the spread of salmonellosis among birds and consumer access to *Salmonella* infected products<sup>13</sup>. Thus, this decree forbade retail trade of infected eggs and slaughtered poultry.

Comparing the share of different DI nosological entities, it was established that SDI and UDI determined ES during the following studied periods 1960-1980, 1981-2000, 2001-2018. The DI profile showed the increase of SDI and UDI share by 1.5 times and amounted to 86.7 % during 2001-2018 compared with 1960-1980.

The share of salmonellosis in 2001-2018 increased by 3.9 times and amounted to 8.9 % compared with 1960-1980s. The share of shigellosis decreased by 9.1 times and amounted to 4.4 %. The share of typhoid fever was insignificant in all studied periods and decreased from 1.1 % in 1960-1980 to 0.01 in 2001-2018.

In 1995 the decree of the Ministry of Health of Ukraine listed especially dangerous, dangerous and parasitic diseases of human which had to be mandatory registered<sup>14</sup>. According to this decree, in addition to cholera; typhoid fever; paratyphoid fever A, B, C; salmonellosis; shigellosis; yersiniosis; campylobacteriosis the medical staff reported the cases of colitis, enteritis, gastroenteritis caused by *Colibacillus*, *Aerobacters*, *Staphylococcus*, *Streptococcus*, *Blue pus bacillus*, *Proteus*, *Klebsiella*, *Enterobacter*, *Enterococcus*, *Serratia*, *Bacteroid*, *Clostridium*, *Rotavirus* and other microorganisms and DI caused by unspecified pathogens of unspecified bacterial food poisoning.

The rise of SDI incidence was established since 2001 (from 60.6 per 100.000 people in 2001 to 81.6 – in 2018, at that in 2010; 2011; 2014 and 2016 the indices were in the range of 102.1-104.4 per 100.000 people). On the contrary, the incidence of UDI decreased from 79.8 per 100.000 people in 2001 to 49.5 in 2018, which indicates an improvement of the laboratory diagnostics of DI.

Ukraine does not officially register DI caused by opportunistic pathogens (OP).

The study, based on the reporting documentation of the health care institutions, established that the incidence of DI caused by *Klebsiella* ranged from 15.7 to 40.2 per 100.000 people, *Enterobacters* – from 3.9 to 24.9 per 100.000 people. *Staphylococcus* – from 13.6 to 20,5 per 100.000 people.

Among the abovementioned DI, only

*staphylococcus* can be considered anthroponosis. *Klebsiella* and *Enterobacter* are ubiquitous in the nature. Taking into account that the main source of *Klebsiella* and *Enterobacter* is soil, the resulting DI can be considered sapronosis. The main route of transmission is through food. OP are characterized by low virulence, and, therefore, to achieve the infecting dose, they need time for reproduction in food. Humans and animals can also be a source of DI pathogens, as these OP are representatives of indigenous intestinal microflora<sup>15</sup>.

Up to this time in Ukraine, the main criterion for the DI diagnostics caused by OP is their detection in the feces or vomit of the patients in the quantity of at least 10<sup>6</sup> CFU per 1 g of feces<sup>16</sup>. That is, the microbiological study at acute intestinal infectious diseases is based on the determination of the quantitative composition of the intestinal microflora and does not take into account the pathogenic potential of microorganisms. In order to identify the pathogenicity factors of OP, a laboratory study was conducted to determine their ALA and ability to adhesion. Lysozyme is a part of the body's immune system and protects it from infectious agents. It is established that OP isolated in etiologically significant doses from the feces of patients with DI, demonstrate ALA in (86.2±3,0) % of cases (table.1).

**Table 1. Antilysozyme activity of OP (M±m) %**

| OP                             | Level of antilysozyme activity |          |          |          |          |
|--------------------------------|--------------------------------|----------|----------|----------|----------|
|                                | 0                              | 4 µg/ml  | 10 µg/ml | 20 µg/ml | 25 µg/ml |
| <i>S. aureus</i><br>(n=50)     | 100                            | 76,0±6,0 | 76,0±6,0 | 52,0±7,1 | 24,0±6,0 |
| <i>K. pneumoniae</i><br>(n=40) | 100                            | 85,0±5,6 | 80,0±6,3 | 80,0±6,3 | 15,0±5,6 |
| <i>E. cloacae</i><br>(n=40)    | 100                            | 100      | 100      | 90,0±4,7 | 10,0±4,7 |

At lysozyme concentrations of 5 and 10 µg/ml, the level of ALA strains isolated from the feces of the patients with acute intestinal infectious disease varied in the range from 76.0 to 100 %. Even at lysozyme concentration of 25 µg/ml in the feces, the microorganisms demonstrated ALA.

It is well known that the adhesion of microorganisms is a releaser in the development of infectious pathological process. The study revealed the ability to adhesion in (50.8±4.4) % of the studied cultures isolated from the feces of patients with DI (Table 2).

**Table 2- OP distribution according to adhesion degree (M±m) %**

| OP                          | Adhesion level |          |          |      |
|-----------------------------|----------------|----------|----------|------|
|                             | adhesive       | low      | moderate | high |
| <i>S. aureus</i> (n=50)     | 36,0±6,8       | 30,0±6,5 | 6,0±3,4  | 0    |
| <i>K. pneumoniae</i> (n=40) | 85,0±5,6       | 75,0±6,8 | 10,0±4,7 | 0    |
| <i>E. cloacae</i> (n=40)    | 35,0±7,5       | 35,0±7,5 | 0        | 0    |

Adhesion is inherent in the prevailing number (85,0 %) of *K. pneumoniae* and in more than one third of *S. aureus* and *E. cloacae* isolated from the feces of the patients with DI. It should be noted that the bacteria with low adhesion dominated in the studied strains.

Consequently, OP ability to overcome the immune defense of the macroorganism, to fix on the surface of enterocytes testifies their significant pathogenic potential, because ALA and the adhesion ability are inherent in OP.

In order to determine the social factors that can determine the EP of DI, the demographics and the access to water supply, sewerage, hot water supply were studied in North-Eastern Ukraine (Sumy Oblast).

It is established that depopulation of region is increasing. The population number decreased from 1,279,900 people in 2003 to 1,094,300 people in 2018 that is by 14.5 %. The number of deaths in 2003-2018 exceeded the number of births by 2.2 times.

Persons older than 18 year dominated the age structure of the population, the number of children decreased constantly. In 2003, there were 249.200 children in Sumy Oblast, but in 2018 – 171.600. That is, the number of children decreased by 31.1 %. There was 1.028.500 adults in 2003, 920.500 - in 2018, or adult population decreased is 10.5 % less. The share of children in the total population composition decreased from 19.5 % in 2003 to 15.7 % in 2018.

Along with the population decrease, the decrease in the population density was also observed. According to the statistics, this index ranged from 53.7 per 1 km<sup>2</sup> in 2003 to 45.9 in 2018.

Vital statistics and migration of the population are important indices of the region demographics. The vital statistics coefficient varied from (-11.4) % in 2003 to (-9.8) % in 2018; the migration coefficient varied from (-3.8) % in 2003 to (-1.9) % in 2009.

Correlation analysis was carried out to verify the

hypothesis that demographics has an impact on the DI incidence. The direct strong correlation between shigellosis incidence and population number, both adults and children (r=0.838, r=0.778 and r=0.872, respectively), the population density (r=0.836); between the incidence of SDI, SDI and UDI and the migration rate of the population (r=0.897 and r=0.736, respectively). Indirect strong correlations were established between the number and density of the population and the incidence of salmonellosis (r=-0.722 and r=-0.721, respectively), SDI ( r=-0.779 and r=-0.779, respectively). That is, if the current trend in demographics remains, when the population decreases from year to year, we should expect a strong increase of both salmonellosis and SDI incidence.

Access of the population to water supply, sewerage, hot water supply is a factor that characterizes the level of social and economic region development. The share of the total area of housing equipped with water supply varied from 44.9 % in 2003 to 50.6 % in 2017, sewerage – from 41.3 % in 2003 to 48.5 % in 2017, hot water supply – from 27.5 % in 2003 to 34.1 % in 2017 in Sumy Oblast.

The correlation analysis found that in addition to the expected indirect correlation between shigellosis incidence and availability of water supply, sewerage, hot water, there was the direct strong correlation between the availability of water supply and sewerage as well as hot water (table. 5).

**Table 3 - Pair correlation coefficients between annual levels of DI incidence and the share of the total area of housing equipped with water supply, sewerage, hot water supply (r)**

| Nosological entity | Living environment |          |                  |
|--------------------|--------------------|----------|------------------|
|                    | water supply       | sewerage | hot water supply |
| Shigellosis        | -0.823             | -0.806   | -0.783           |
| Salmonellosis      | 0.729              | 0.751    | 0.743            |
| SDI and UDI        | 0.736              | 0.726    | 0.758            |
| SDI                | 0.852              | 0.872    | 0.851            |
| UDI                | -0.264             | -0.295   | -0.275           |

The abovementioned indirectly points to the issues existing in the services provision for the population, namely, microbially contaminated drinking water.

Consequently, the following social (decreased role of a human as an infection source, increased role of animals and soil as OP source; depopulation, less than half of the housing is equipped with water supply, sewerage, hot water supply) and biological (OP demonstrate ALA and adhesion ability) factors have changed EP of DI, namely they decreased the

incidence of intestinal anthroponoses (shigellosis, typhoid fever) and increased intestinal zoonoses (salmonellosis) and sapronoses (DI caused by OP).

**Conclusions:**

1. ES with DI is characterized by a sharp decrease in the incidence of typhoid fever and shigellosis, increase in the incidence of salmonellosis and dominance of SDI and UDI in the nosological structure ( $p < 0.05$ ).
2. The incidence of DI caused by Klebsiella, Staphylococcus, Enterobacter exceeds the incidence of salmonellosis, shigellosis, typhoid fever ( $p < 0.05$ ). OP, which are DI pathogens, have ALA and adhesion ability, which proves their pathogenic potential.
3. The demographics indirectly affects the DI incidence. The correlations have been found between population number and the incidence of shigellosis ( $r = 0.838$ ) and salmonellosis ( $r = -0.722$ ), SDI ( $r = -0.779$ ); population density and shigellosis ( $r = 0.836$ ), salmonellosis ( $r = -0.721$ ), SDI ( $r = -0.779$ ); migration and SDI and UDI ( $r = 0.736$ ).

4. The access level to water supply, sewerage, hot water supply correlates with the incidence of DI. Correlations between the abovementioned factors and the incidence of shigellosis ( $r =$  from  $-0.783$  to  $-0.823$ ), salmonellosis, SDI and UDI (from  $r = 0.729$  to  $0.872$ ) have been established.
5. The system of ES over DI in Ukraine needs improvement by optimisation its information and analytical subsystems aimed at objective epidemiological diagnostics and ES forecasting.

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**Autors's contribution:**

Data gathering and idea owner of this study: Nina Malysh, Alla Podavalenko, Svitlana Birukova,

Study design: Nina Malysh, Mykola Chemych, Viktoriya Zadorozhna

Data gathering: Nina Malysh, Alla Podavalenko, Viktoriya Zadorozhna,

Writing and submitting manuscript: Nina Malysh, Alla Podavalenko,

Editing and approval of final draft: All authors

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