



Serotype and genotyping of *Salmonella* sp. isolated from street foods in N'Djaména, Chad

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ABSTRACT

Group foodborne illness infections with *Salmonella* are often caused by the simultaneous consumption by several people of heavily contaminated food. The objective of this work was to determine the phylogenetic relationships between dietary strains of *Salmonella* sp. isolated from our samples and confirmed clinical strains. A total of 447 samples were collected and analyzed using standard food microbiology methods. Enterobacterial Repetitive Intergenic Consensus-Polymerase chain Reaction (ERIC-PCR) technique allowed confirmation that all our strains belong to the genus *Salmonella*. From ten (10) strains characterized, eight (8) different genotype profiles were identified. The different DNA fragments of the isolates were generated by ERIC-PCR and then revealed on the agarose gel and had bands ranging from 2 to 6 bands with sizes between 150 and 1500 bp. The two food strains of *S. Mbadaka* (1 and 2) presented identical profiles. However, it is noted that two (2) strains of *S. Anatum* had different ERIC-PCR profiles. There is also a very high resemblance between the strain of *S. Anatum* (strain 5) and the confirmed clinical strains (H5: *Salmonella* Typhi). The presence of risky germs in the food analyzed poses a public health problem and should call on the competent authorities and prompt the taking of corrective measures.

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Keywords: Ready-to-eat, street food, Molecular characterization, *Salmonella* sp.

INTRODUCTION

Street food is defined as food and beverage prepared and / or sold by vendors in streets and other public places for immediate consumption or for subsequent consumption

without further processing or preparation (FAO, 1988 ; Barro et al., 2008).

Food handlers play a critical role that can positively or negatively influence food safety. It has been revealed in some studies that

the lack of training of these producers could be a compromising element for the safety of foods offered on public roads (Hilario, 2015 ; Cortese *et al.*, 2016).

Studies conducted in Burkina Faso, Ghana and Nigeria have clearly revealed that these foods are sold near open gutters, with the presence of stray animals and even insects which are real vectors of contamination (Barro *et al.*, 2002 ; Nurudeen *et al.*, 2014 ; Hilario, 2015 ; Ntow *et al.*, 2016). The main causes of the unhealthy nature of these foods come from non-compliance with good hygiene practices in their processing, cooking, preservation or sale (Mufizur *et al.*, 2014 ; Tidjani *et al.*, 2016 ; Ire, 2016). Food handlers are the most common source of contamination, as they can spread pests through dirty hands or skin lesions, as well as dirty cookware or kitchen counters (Linscott, 2011). Bacterial agents are a source of contamination of many food products (Guiraud, 2012).

Salmonellosis comprises two main types of infections : on the one hand, typhoid fever and paratyphoid fevers and on the other hand non-typhoidal (or non-typhoid) salmonellosis. According to the World Health Organization (WHO, 2013), *Salmonella* (non-typhoid) is responsible for tens of millions of cases of foodborne and waterborne illness in humans worldwide each year. These diseases have a significant negative economic impact in resource-limited countries amounting to \$ 110 billion per year (Jaffee *et al.*, 2018).

In Chad, Bessimbaye *et al.* (2013) in their study showed that most diarrheal diseases are due to coliforms and especially pathovars of *E. coli*, *Salmonella enterica* and *Shigella*, Mayoré *et al.* (2021), show the occurrence and antimicrobial resistance of *Salmonella* sp. from street food collected in Chad.

The conception of the correspondences between different serovars of *Salmonella* and their genotypes diversity is important knowledge about the diversity of *Salmonella* strains circulating in Chad. Indeed, among genomic methods often used for genotype diversity analysis, we have Enterobacterial Repetitive Intergenic Consensus, PCR (ERIC-PCR). ERIC-PCR has the ability to

discriminate between strains of the same or closely related species (Bakhshi *et al.*, 2018 ; Aljindan *et al.*, 2018 ; Kumar *et al.*, 2019).

The aim of this study was to provide scientific data on molecular diversity of *Salmonella* isolated from street foods in N'Djamena, using ERIC-PCR.

MATERIALS AND METHODS

Site and period of study

The study took place in N'Djamena, the political capital of Chad between October 2014 and January 2018. Of the 10 districts in N'Djaména, eight (1, 2, 3, 4, 5, 6, 7 and 9) were selected for this study.

Sample's collection

Four hundred and forty-seven (447) food samples were purchased from specially targeted sites (Figure 1). Five hundred (500) g (for solid products) and five hundred (500) ml (for liquid products) were collected in sterile sachets or vials, and transported to the laboratory in a + 4°C cooler for microbiological analysis within two hours.

Random sampling included the ground beef sandwich (n = 42); the "ball" with okra sauce (n = 42); rice with sorrel sauce (n = 39); rice with tomato sauce (n = 36); grilled mutton (n = 49); fried fish (n = 31); banana juice (n = 31); avocado juice (n = 31); raw mutton (n = 41); raw beef (n = 44); raw fish (n = 33) and grilled mutton ingredients (n = 28).

Microbiological analysis

Salmonella strains were isolated from four hundred and forty-seven (447) samples, according to the ISO 7218 standard.

Pre-enrichment in non-selective broth

A quantity of 25 g (solid product) or 25 ml (liquid product) of samples was homogenized in 225 ml of buffered peptone water (Liofilchen, Italy) and incubate at 37°C for 16 to 20 hours.

Selective enrichment in broth

Following the non-selective pre-enrichment stage, 1 ml was transferred in Rappaport-Vassiliadis (41.5°C) and Muller-Kauffmann broths with tetra thionate-

novobiocin (37°C) and the incubated for 18 to 20 h.

Selective isolation

A loopful of culture suspension from each selective media was placed on two different agar plates to identify individual colonies.

Selective media used for this were Xylose Lysine Desoxycholate (XLD) and Hektoen agar media. The colonies presenting the typical appearance of *Salmonella* were subjected to identification tests namely the use Kligler-Hajna agar which consisted of looking for a slope (red), a pellet (yellow), with formation of gas and hydrogen sulphide (darkened agar). The second test which was biochemical was the use of urea-indole medium (urease negative and indole negative). The presumed *Salmonella* colonies were confirmed by the API 20E gallery (BioMérieux, France).

Serotyping of *Salmonella* sp.

Serotyping of *Salmonella* strains was performed by the direct slide agglutination technique with the combination of Ag O (wall) and Ag H (flagellum) antigens according to the Kauffmann-White diagram (Kauffmann, 1966). The *Salmonella* strains were checked to ensure that they are not in the R phase, that is, a phase where self-agglutination occurs. Serotypes were determined using the combination of "O" and "H" antigens obtained by agglutination test on pure culture isolated for 24 hours on non-selective agar. For the strains on which auto-agglutination was not detected were successively tested with O-polyvalent (OMA, OMB and OMC), O-monovalent and H (HMA, HMB, HMC and H1) according to the Kauffmann-White diagram (Kauffmann, 1966). Generally, the polyvalent sera OMA and OMB are supposed to allow the determination of 99% of *Salmonella* strains. The specification of the group to which the *Salmonella* strains belong was made effective through the use of Omonoalant antisera.

To determine the antigenic formula and the reading of the serotyping results, the Kauffman-White minor table we used

(Grimont *et al.*, 2007 ; Guibourdenche *et al.*, 2010).

Genotyping of *Salmonella* strains using ERIC-PCR.

An Enterobacterial Repetitive Intergenique Concensus-Polymerase Chain Reaction (ERIC-PCR) was carried out as a genetic marker in order to allow us to reconcile the phylogenetic profiles and possibly the clonal links of the strains between them. For the rating of the sizes of the DNA fragments, co-migration of the fragments using the 100 marker with bands ranging from 100 base pairs (bp) to 3000 bp was performed.

Genomic DNA extraction

The extraction of DNA was carried out by thermal choc technique using overnight culture both. A total volume of 1.5 ml of the broth was taken into an Eppendorf tube, centrifuged at 12,000 rpm for 10 minutes, and the supernatant carefully removed. The precipitate was subsequently resuspended in 100 µl of PBS (Phosphate buffered saline) and centrifuged again at 12000 rpm for 10 min to wash the cells of the medium. The supernatant was again removed, the precipitate dissolved in 100 µl of distilled water was brought to the boil for 10 min then centrifuged one last time at 12,000 rpm for 10 min to separate the DNA from the other constituents of the bacteria. Finally, the supernatant, gently taken into another Eppendorf tube and stored at -20° C was used as DNA for the amplification reactions. For verification of the extracted DNA, gel electrophoresis was performed with 1% agarose.

Genomic DNA Amplification

The technique is based on the polymerase chain reaction or PCR (Polymerase chain reaction), it allows the in vitro amplification of DNA sequences by enzymatic synthesis. The technique was automated using an enzyme (Taq DNA polymerase), thermostable, isolated from a thermophilic bacterium (*Thermus aquaticus*), adapted to life in hot springs (Saiki *et al.*, 1988), in the presence of specific primers and nucleotides (dNTPs). Chromosome profiles by amplification were determined and compared

by ERIC-PCR (Enterobacterial Repetitive Intergenic Consensus-Polymerase chain Reaction).

Controls strains

For all the amplification reactions, the Negative Control (TN) was constituted by the reaction mixture and ultra pure water. The Positive Control (TP: *Salmonella* Typhi) and the rest of the confirmed clinical strains (H1:

Salmonella Hadar; H2: *Salmonella* Limete; H3: *Salmonella* Anatum; H4: *Salmonella* Paratyphi A; H5: *Salmonella* Typhi) were generously provided by the Laboratory of Bacteriology of the General National Reference Hospital (HGRN) of N'Djaména where they were serotyped and confirmed by Vitek® 2 Compact 15.

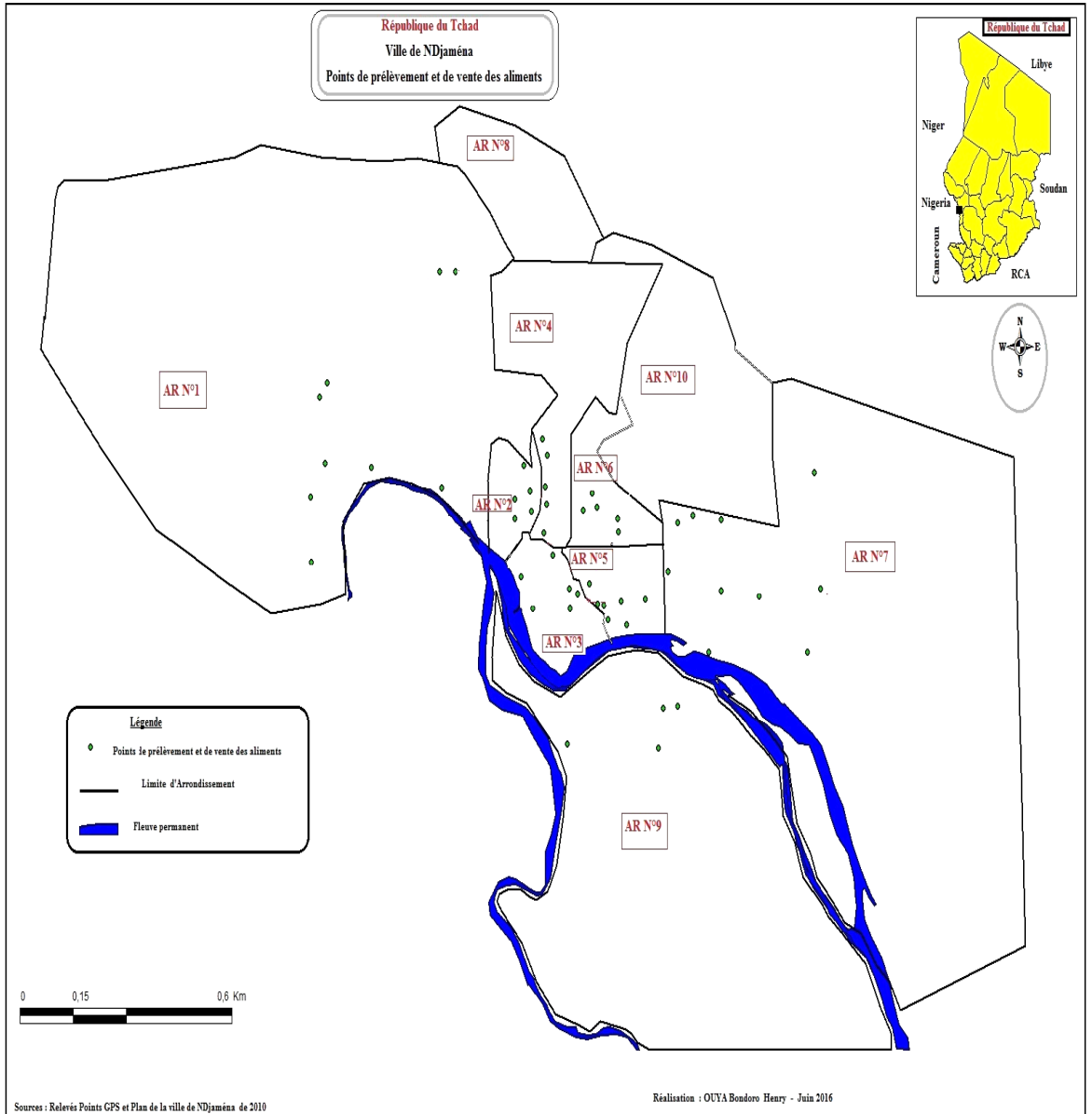


Figure 1 : Map of N'Djaména showing sampling sites.

RESULTS

Prevalence of *Samonella* sp. in analyzed foods

From the 447 samples, a total of 12 different types of food have been analyzed. The presence of *Salmonella* sp. has been observed in 5 samples, these samples were rice with sorrel sauce, thus giving a contamination rate of 12.82% for this type of food (Table 1).

Serotyping

Serotyping of strains allowed to identify 3 different serotypes, (02) *S. Mbandaka*, (01) *S. Idikan*, (02) *S. Anatum* (Table 2).

Molecular characteristics of *Salmonella* sp. isolated rice with sorrel sauce.

Ten (10) strains of *Salmonella* sp. were characterized by ERIC-PCR, including 5 strains from collected food samples (numbered 1 to 5 on the images) and 5 confirmed clinical

strains (numbered H1 to H5 on the image) obtained from "Hôpital Général de Référence Nationale de N'Djaména" for comparison (Table 3). From the 10 *Salmonella* sp. strains, 8 distinct ERIC-PCR profiles were identified. The different DNA fragments of the isolates generated by ERIC-PCR and then revealed on the agarose gel had bands ranging from 2 to 6 bands with sizes between 150 and 1500 bp.

Uppercase Roman numerals (I through VIII) was used to identify identical ERIC-PCR genotype profiles. The table below (table 3) presents the synthesis of the profiles obtained by ERIC-PCR. The 2 strains of *S. Mbandaka* presented identical ERIC-PCR profiles or 2 strains of *S. Anatum* had different ERIC-PCR profiles. There was also a very high resemblance between the strain of *S. Anatum* (strain 5) and the confirmed clinical strains (H5: *Salmonella* Typhi).

Table 1: Prevalence of *Samonella* sp. in analyzed foods.

Type of food	Frequency (%)
Minced Beef Sandwich (n = 42)	-
"Ball" with okra sauce (n = 42)	-
Rice with sorrel sauce (n = 39)	05 (12.82 %)
Rice with tomato sauce (n = 36)	-
Grilled mutton (n = 49)	-
Fried fish (n = 31)	-
Banana juice (n = 31)	-
Avocado juice (n = 31)	-
Raw mutton (n = 41)	-
Raw beef (n = 44)	-
Raw fish (n = 33)	-
Grilled mutton seasoning (n = 28)	-
Total (n = 447)	05 (1.12 %)

- : negatif

Table 2: *Salmonella* sp. Serotypes isolated samples of rice with sorrel sauce.

Antigenic formula	Serotype	Number
6,7: z ₁₀ e,n,z ₁₅	<i>S. Mbandaka</i>	02
1,13,23: i 1,5	<i>S. Idikan</i>	01
3,10: e,h 1,6	<i>S. Anatum</i>	02

Table 3: Summary of the different profiles of *Salmonella* strains isolated from rice in sorrel sauce and of the reference strains.

Reference	Origin	Serotype	ERIC-PCR
1	Rice in sorrel sauce	<i>S. Mbandaka</i>	I
2	Rice in sorrel sauce	<i>S. Mbandaka</i>	I
3	Rice in sorrel sauce	<i>S. Idikan</i>	II
4	Rice in sorrel sauce	<i>S. Anatum</i>	III
5	Rice in sorrel sauce	<i>S. Anatum</i>	IV
H1	Clinical sample	<i>Salmonella</i> Hadar	V
H2	Clinical sample	<i>Salmonella</i> Limete	VI
H3	Clinical sample	<i>Salmonella</i> Anatum	VII
H4	Clinical sample	<i>Salmonella</i> Paratyphi A	VIII
H5	Clinical sample	<i>Salmonella</i> Typhi	IV

DISCUSSION

The majority of foods analyzed indicate the absence of *Salmonella*, this result is consistent with those of many studies conducted on street foods (Kauffmann et al., 2006 ; El Marnissi et al., 2012). This absence is not a guarantee of the good quality of the food sold and does not completely exclude the presence of *Salmonella* because the effect of competition can also prevent *Salmonella* from being expressed. In street foods, there is a strong presence of *Salmonella* as in several other studies (Barro et al., 2002 ; Christison et al., 2008 ; Ahoyo et al., 2010). This presence could come from the sorrel leaves which are used for the preparation of the sauce if the cooking is not well done or from cross-contamination from poorly washed utensils. In food, the presence of *Salmonella* is worrying for the health of the consumer. *Salmonella* is

the main cause of foodborne illness and the factors favoring its presence are generally the sales environment and equipment (Belomaria et al., 2007). The different serotypes identified in rice with sorrel sauce, namely *S. Mbandaka*, *S. Idikan* and *S. Anatum* were also found (Shafini et al., 2017 ; Da Cunha-Neto et al., 2017). *S. Anatum* is frequently detected in cattle and has been frequently detected in faeces, skin, lymph nodes, meat fluids, and carcasses of dairy and beef cattle in the southern United States (Kunze et al., 2008), as in the case of beef from Mexico (Varela-Guerrero et al., 2013), Namibia (Shilangale et al., 2015) and South Africa (Madoroba et al., 2016). In France for example, between 2007 and 2012, the data collected on the food samples analyzed underline an almost constant change in the number of strains of the *S. Mbandaka* serovar (Renaud et al., 2015).

S. Mbandaka strains (1 and 2) present an identical profile, which suggests a close genetic relationship between them (figure 2). Previous studies on ERIC models have indicated species-specific and also strain-specific profiles (Versalovic et al., 1991 ; Kerouanton et al., 1996 ; Millemann et al., 1996 ; Burr et al., 1998). However, the two strains of S. Anatum gave two different profiles (III and IV) (Figure 2). ERIC-PCR has been shown to be a powerful tool to demonstrate variability between isolates (Sachdeva et al., 2004 ; Fendri et al., 2013). A study also reported that the

ERIC-PCR technique would be of interest for the intraserotypic typing of *Salmonella* strains (Kumao et al., 2002). Although this has not yet been demonstrated, it has been suggested that ERIC sequences are likely to have a functional role in bacterial cells (Asinimov et al., 2005 ; De Gregorio et al., 2005).

The IV profile identical to the food strain (strain 5) and to the confirmed clinical strain (H5) raises real concern as it could explain a link between the *Salmonella* strains found in food samples and those in clinical samples (Figure 3).

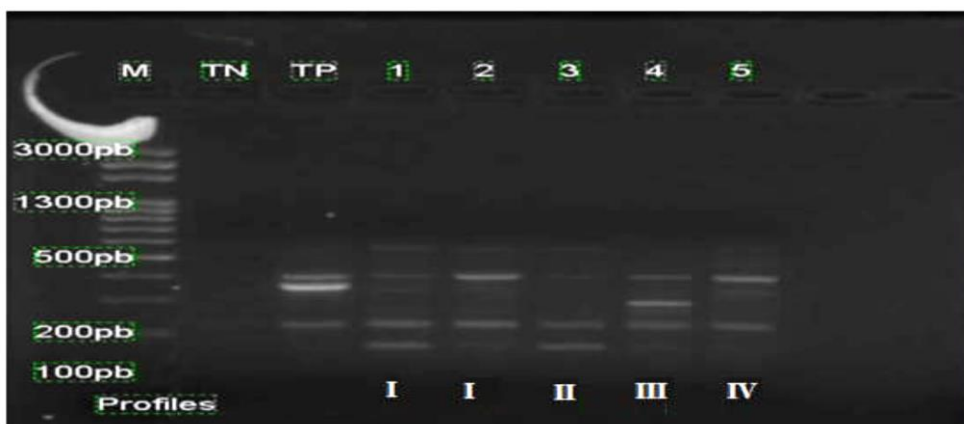


Figure 2: ERIC-PCR profiles of *Salmonella* strains isolated from rice in sorrel sauce.

Legend : pb : base pairs ; M : Weight Marker 100 ; TN : Negative Witness ; TP : Positive Witness (*Salmonella* Typhi) ; 1 : S. Mbandaka ; 2 : S. Mbandaka ; 3 : S. Idikan ; 4 : S. Anatum ; 5 : S. Anatum.

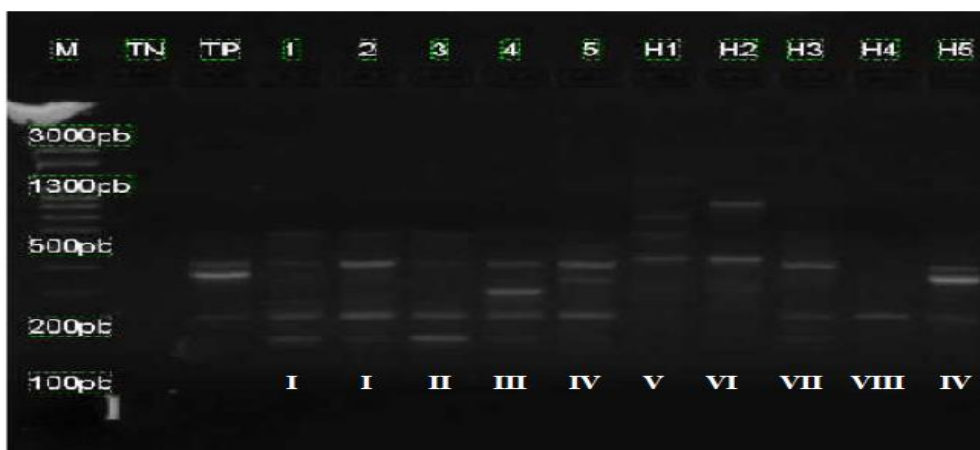


Figure 3: ERIC-PCR profiles of *Salmonella* strains isolated from rice in sorrel sauce and confirmed clinical strains.

Legend : pb : base pairs ; MP : Weight Marker 100 ; TN : Negative Witness ; TP : Positive Witness (*Salmonella* Typhi) ; 1 : S. Mbandaka ; 2 : S. Mbandaka ; 3 : S. Idikan ; 4 : S. Anatum ; 5 : S. Anatum ; H1 : S. Hadar ; H2 : S. Limete ; H3 : S. Anatum ; H4 : S. Paratyphi A ; H5 : S. Typhi.

Conclusion

The presence of three *Salmonella* serotypes in our study reveals the possibility of cross-contamination from the environment and poor hygiene during the process of preparing and selling street food. PCR-ERIC was used successfully and identified a total of 8 genotypes from the ten *Salmonella* isolates from food and confirmed clinical strains. The health risks associated with street foods could be minimized if good hygienic practices were observed when preparing, storing and selling these foods.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

ADM: laboratory analysis, data processing and analysis, preparation of the first draft of the article. AB: laboratory analysis, data processing and analysis, preparation of the first draft of the article. EB: preparation of the first draft of the article. NB: preparation of the first draft of the article. RG: laboratory analysis. AT: proofreading and correction of the article. NB: proofreading and correction of the article.

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