عزل وتشخيص بعض أنواع البكتريا المسببة للالتهابات الشديدة لدى الأطفال حديثي الولادة

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الخلاصة
عادة ما تكون الالتهابات التي سببها البكتريا ما يعرف اصطلاحا بالخمجات الجرثومية شائعة عند الأطفال، وخاصة الأطفال الرضع وحديثي الولادة. مكمن الخطورة في هذه الالتهابات عند الأطفال من أنها تتحمل نسبة عالية من المضاعفات المميتة للأطفال، مثل تسمم الدم أو التهاب السحايا في حالة تركها دون علاج. في أغلب الأحيان، يكون تشخيص هذه الالتهابات الجرثومية سريرياً إضافة إلى التشخيص المختبري لتحديد نوع البكتريا السبب للالتهاب في بعض الحالات. ويتم البياتي بناءً على الانتهاك الجرثومي الخطير مثل التهاب المفاصل البولية والالتهابات الجهاز الهضمي والрезاز من الدم عند الأطفال الرضع والحديثي الولادة في الجانب الأيمن في مدينة الموصل، ودراسة وحذف المعايير المحيطة بالمديرية البيطرية المشتركة من المعايير البيطرية للمواطنين. فالمراقبة، ودراسة وحذف مدى تأثيرها على المضادات الحيوية المختلفة. تتم دراسة عينات مختلفة من الدم والادرار (27) عينة وعينات الدم (6) اخترعت من الأطفال الحديثي الولادة والداخلين إلى مستشفى الموصل العام في الجانب الأيمن من مدينة الموصل في الفترة الممتدة بين 1/7/2018 وال1/9/2018. أشارت نتائجنا إلى أن العظام العنقودية الذهبية كانت العظام الجرثومية الأكثر شيوعا في الدم (81٪)، حيث أن كل من العظام العنقودية الذهبية والبيكروكولوكولوكولوغرافية كانت الأكثر شيوعا في العينات المحاذية من الدم (48٪ و 43٪ على التوالي). بتبعها كل من الممتلئات والرخاف الزنانية (4.5٪ لكل منهما). أثبتت الدراسة أن كل المراقبة العنقودية الذهبية والبيكروكولوغرافية كانت حساسة للغاية لمادة أموكسيسيلين / حمض كلوفاناميك (83٪) والنيفوكلوسيسين (88٪) للكولوكولوغرافية والبيكروكولوغرافية. مع ذلك، كلاهما ممتازاً للغاية لتسليب السولونجيلين (100٪) والسيفالوسبورينات والمربيتيين (100٪). علاوة على ذلك، فإن البكروكولوغرافية غير فعالة للعظام العنقودية. وحصصت في الحالات شديدة المقاومة للتمييز ظهور مقاومة مبكرة للمضادات الحيوية.

الكلمات المفتاحية: الجانب الأيمن لمدينة الموصل, الالتهابات الجرثومية, بين الأطفال حديثي الولادة
Isolation and Identification of Some Bacteria from Severe Infections Among Children in the West Bank of Mosul City

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Abstract

Infections caused by bacteria are common in infants and neonates. Some of these infections are severe and usually carry high risk of fatal complications such as septicemia and meningitis if left un-treated. Very often, the diagnosis of these infections is clinical as well as laboratory diagnosis to determine the type of bacteria in body fluids such as urine, stool, blood and CSF. The present study aims to isolate and identify the bacteria responsible for some types of severe infections in neonates such as urinary tract infections (UTI), gastrointestinal tract (GIT) infections and bacteremia in West bank of Mosul city and test their susceptibility to different antibiotics. Twenty-seven (27) samples from blood, 28 from urine and 6 from stool were collected from neonates admitted to "Mosul General Hospital" in West Bank of Mosul city in the period between 1/7/2018 to 1/9/2018. Our results indicated that Staphylococcus aureus was the most common bacterial isolate in blood (81%), whereas both Staphylococcus aureus and Esch. coli were common in urine (48% and 43% respectively) followed by Proteus and Pseudomonas aeruginosa (4.5 % each). Both Staphylococcus aureus and Esch. coli were highly sensitive to amoxiclav (83% for and 100% respectively), levofloxacain (88% for Staphylococcus aureus and 100 % for Esch. coli) and meropenem (100% for both Staphylococcus aureus and Esch. coli). However, both of them were highly resistant to ampicillins (100%) and 3rd generation cephalosporins. Although meropenem is effective, it's use should be selected and restricted to highly resistant cases to avoid the emergence of early antibiotic resistance.

Keywords: West Bank of Mosul, Bacterial Infections Among Children, Antibiotics.
Introduction

Children are prone to different types of infections including viral, bacterial, parasitic and fungal infections(1). Although viral infections are more common than others, most of them are self-limiting and go even without treatment(2) . On other hand, bacterial infections usually need treatment and many viral infections might be complicated with secondary bacterial infections(3) .

Among children, the most common bacterial infections are throat infections (usually caused by Streptococcus pyogenes and Haemophilus influenzae), ear infections and sinusitis (Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis) and conjunctivitis (Saphylococcus aureus, H influenzae, S pneumoniae, and M catarrhalis(4)). Skin infections, GIT infections and UTI are also regularly seen in children(4). The diagnosis of these infections is usually clinically. However, in certain other infections, bacteria must be identified in different samples depending on site of infection including tissues, blood, stool, and body fluids like urine and CSF. Identification of bacteria in these samples can be done either microscopically, by culture, or by using different biochemical tests or molecular methods.

Bacterial infections whether in children (or adults) are usually treated with antibiotics(5) . Unfortunately, due to mal-prescription of these antibiotics, bacterial resistance to different antibiotics is now a common problem world-wide(5,6,7). Susceptibility of bacteria to different antibiotics is commonly tested in vitro by antibiotic sensitivity test using antibiotic sensitivity disks(8) .

Infants and Children between the age of 1 and 36 months are particularly prone to bacterial infections including occult bacteremia with probable subsequent development of more serious infections such as sepsis and meningitis(9,10,11,12) because of their low immunity since their immune system is not fully developed yet(1). In these children, empirical treatment with antibiotics is frequently practiced in an attempt to reduce / prevent deaths. In resistant cases determination of antibiotic sensitivity in theses patient is often necessary(10).

The aim of current study is to isolate different bacteria responsible for some of more severe bacterial infection in neonates by collecting samples from different body locations, diagnosing them by conventional bacteriological methods, and testing them for antibiotic sensitivity by antibiotic sensitivity test.

Materials and Methods

Using clean aseptic bacteriological techniques, twenty seven (27) clinical samples from the blood, 28 from the urine and 6 samples from the stool were collected from 61 neonates recently attending Mosul General Hospital in the West bank of Mosul city ranging in age between 1 day and 1 month during the period between 1/7/2018 to 1/9/2018. Two ml of blood samples were drawn by their puncture and transferred into brain heart infusion broth. Urine and stool samples were collected in sterile containers and transported within a short period of time to "Microbiology Lab." at hospital or "Teaching laboratory” at College of Science / University of Mosul where the isolation of bacteria, identification of their spp., and determination of their antibiotic sensitivity were done using conventional bacteriological methods and antibiotic sensitivity test.
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respectively. In brief, the samples were first cultured on different bacteriological media including nutrient agars, blood agars, and MacConkey agars. The cultures were first incubated at 37 °C for 24 hours for primary cultivation. On the day after, the petri dishes were tested for the presence of bacterial growth, and identification of the bacteria were done by using conventional bacteriological methods. Special media such as mannitol salt agar were used to aid in the diagnosis of microorganisms(13).

Antibiotic Sensitivity Test

The isolates were tested for their antimicrobial susceptibility to following antibiotics using Kirby – Bauer antibiotic disc diffusion method(13,14): Ampicillin (10 µg), Ceftriaxone (30 µg), Cefotaxime (30 µg), Amoxiclav (30 µg), Norfloxacin (10 µg), Erythromycin (15 µg), Chloramphenicol (30 µg), Meropenem,(10 µg), Levofloxacin (5 µg), Azithromycin (15 µg) and Nalidixic acid (30 µg). These antibiotic disks were available commercially and purchased from local markets. Using a fresh and pure culture, a suspension of 0.1 ml of 0.5 McFarland Standard were transferred over the entire area of Mueller Hinton agar (MHA). With a sterile forceps the antibiotic discs were placed onto the inoculated MHA plate, ensuring sufficient space between individual discs to allow for proper measurement of inhibition zones. The plates were then incubated at 37°C for 18-24 hours. After incubation, the areas of inhibition around the disks (clear areas) were measured by a ruler, recorded in mm and labeled as sensitive (S) and resistant (R). The results were interpreted and compared to standards according to CLSI(14) (Clinical and Laboratory Standards Institute guidelines (2000))

Results

Samples distribution according to gender and age

The distribution of study samples according to gender is illustrated in (Figure 1). Out of the sixty one (61) collected samples, seventeen (27.9%) were males whereas forty four (72.1%) were females.

According to age distribution, all the study samples were taken from children under 1 month of age (neonates). However, for descriptive purposes the samples were subdivided into those who are under , and those who are older than 2 weeks of age. Overall, thirty – four (55.7.3%) neonates were under 2 weeks of age in comparison to 27 (44.3%) older than 2 weeks (Figure 2).
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Figure 1: Gender distribution of study samples

Figure 2: Sample distribution by age

Isolation and identification of bacteria in different samples

Culturing of clinical samples on blood, nutrient and MacConkey agars has revealed positive growth in 50 samples among the total 61 clinical specimens, constituting about 82% of total samples (Table 1). These include 21 (77.7%) positive cultures for blood, 23 (82%) for urine and 6 (100%) for stool samples respectively. On other hand, only 11 samples (18%) were proved to be negative on culture, including 6 blood (22.2%) and 5 urine samples (17.8%). Following isolation, identification and determination of bacterial isolates was carried out based on morphological characteristics (gram stain), cultural properties and biochemical tests. Among blood
isolates, *Staphylococcus aureus* was the most frequent isolated bacteria (81%) followed by *Escherichia coli* (19%). Regarding positive urine cultures, *Staphylococcus aureus* was again the commonest isolate (48%) followed by *Esch. coli* (43%), and *Proteus* and *Pseudomonas aeruginosa* (4.5% each). Not surprisingly, all the 6 stool cultures were positive for *Esch. coli* (100%). Over all, *Staphylococcus aureus* were the commonest isolate among all study samples (28 isolates) followed by *Esch. coli* (20 samples) respectively (Figure 3).

Table 1: Results of bacterial cultures, isolation and identification

<table>
<thead>
<tr>
<th>Sample</th>
<th>Positive cultures</th>
<th>Negative cultures</th>
<th>Bacterial Isolate</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Blood (27)</td>
<td>21</td>
<td>77.8</td>
<td>6</td>
<td>22.2</td>
<td>17</td>
</tr>
<tr>
<td>Urine (28)</td>
<td>23</td>
<td>82.2%</td>
<td>5</td>
<td>17.8</td>
<td>11</td>
</tr>
<tr>
<td>Stool (6)</td>
<td>6</td>
<td>100%</td>
<td>/</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Total (61)</td>
<td>50</td>
<td>82</td>
<td>11</td>
<td>18</td>
<td>50</td>
</tr>
</tbody>
</table>

Figure 3: Frequency of bacterial isolates in all study samples
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Antibiotic sensitivity test

The result of antibiotic sensitivity test is shown in (Table 2). All the Staphylococcus aureus isolates were 100 % resistant to ampicillin, ceftriaxone and erythromycin. However, they were 100 % sensitive to meropenem, and, to lesser extent, to levofloxacin (88%) and amoxiclav (87%) respectively. All other tested antibiotics including cefotaxime, norfloxacin, nalidixic acid and azithromycin were less effective against Staphylococcus aureus with a sensitivity rate ranging from 17-33%. Regarding Esch.coli, our results indicated that all the isolates were resistant to ampicillin, cephalosporins, erythromycin and nalidixic acid with 100% resistant rate. On the other hand, all the isolates were 100% sensitive to norfloxacin, meropenem, amoxiclav and levofloxacin. In addition, chloramphenicol is also highly effective against Esch.coli with 80 % sensitivity rate while azithromycin is only 33% effective. Moreover, Pseudomonas aeruginosa is 100 % resistant to all 11 tested antibiotics.

Table 2: Antibiotic sensitivity of different isolates

<table>
<thead>
<tr>
<th>Antibiotic tested</th>
<th>Staph.aureus</th>
<th>Esch.coli</th>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Ampicillin (AM)</td>
<td>100%</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Ceftriaxone (CRO)</td>
<td>100%</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Cefotaxime (CTX)</td>
<td>67%</td>
<td>33%</td>
<td>100%</td>
</tr>
<tr>
<td>Amoxiclav (AMC)</td>
<td>14%</td>
<td>86%</td>
<td>0%</td>
</tr>
<tr>
<td>Norfloxacin (NX)</td>
<td>67%</td>
<td>33%</td>
<td>0%</td>
</tr>
<tr>
<td>Erythromycin (E)</td>
<td>100%</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Chloramphenicol (C)</td>
<td>67%</td>
<td>33%</td>
<td>20%</td>
</tr>
<tr>
<td>Meropenem, (MR)</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Levofloxacin (LEV)</td>
<td>12%</td>
<td>88%</td>
<td>0%</td>
</tr>
<tr>
<td>Azithromycin (AZM)</td>
<td>83%</td>
<td>17%</td>
<td>67%</td>
</tr>
<tr>
<td>Nalidixic acid (NA)</td>
<td>67%</td>
<td>33%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Discussion

Severe bacterial infection in neonates is a major burden in low and middle income countries especially in first 24 hours after birth where mortality rate is very high(15). In these contexts, early diagnosis and treatment is crucially important to avoid the development of antibiotic resistance and subsequent possible deaths(16). Unfortunately, diagnosis of severe bacterial infections in neonates is often challenging because there is no universally accepted standard definition for it(17). In addition, access to more advanced techniques used in diagnosis such as molecular methods in...
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poor - middle income countries is usually limited and mainly confined to research setting(18).

In Iraq as in many other 3rd world countries, diagnosis of severe bacterial infections depends on both clinical and microbiological criteria. In this research we tried to highlight some of the most complicated neonatal infections in Mosul city with risk of bacteremia development such as urinary tract infections (UTIs) and gastroenteritis. These infections carry risk of subsequent development of severe bacterial infections like sepsis and meningitis(19,20). Therefore, the bacteria were isolated from blood, urine and stool.

The results of the current study indicated that Staphylococcus aureus were the most common bacterial isolate in blood with 81 % isolation rate (17 out of 21 positive samples) followed by Esch.coli (4 samples, 19%). In fact, these results are not surprising and are comparable to the results of other researchers in the developing countries including our country(21,22,23,24). Possible explanation include nosocomial infections, canulation, presence of central venous and urinary catheterization(25). The picture is not far different from the adults where Staphylococcus aureus is the leading cause of bacteremia / septicemia among hospitalized patients in adults(26).

Regarding urine isolates, Staphylococcus aureus were again the most common bacteria isolate ( 11 samples, 48%) followed by Esch.coli (10 samples, 43%) , Proteus and Pseudomonas aeruginosa ( 1 sample, 4.5% each) respectively. These results are somewhat mis-understandable since Staphylococcus aureus is relatively uncommon cause of UTI in infants and children(27). Most of other research revealed that Esch.coli is the most common bacterial cause of UTI in infants(28,29,30,31,32). These discrepancies between the result of the current study and the results of other researchers can be explained on the bases of different sample size and different geographical locations. While these pictures might reflect the real prevalence of Staphylococcus aureus in urine among infants in Mosul city, it is more likely to be either contaminants due to poor hygiene or statistical bias due to small sample size included in this study. However, Staphylococcus infection in urine of infants should always rise the suspicion of the presence of urinary tract anomalies(33).

Studying stool samples had revealed that Esch.coli were isolated from all 6 studied stool samples in infants with gastroenteritis. Esch.coli gastroenteritis in infants is rare where rotavirus (RV) is responsible for up to 87% of infantile diarrhea(34). Esch.coli is a normal inhabitants of the colon, and therefore, determination of the clinical significance of this isolation depends on the determination of whether the isolated strains were pathogenic or not. Identification of pathogenic Esch.coli needs either serotyping which is easy, cheap and available or more sophisticated techniques like molecular methods. However, due to lack of our resources in this study, no determination of pathogenicity of Esch.coli was made. Moreover, viral studies are needed to exclude a viral cause for diarrhea in these infants with gastroenteritis. Taking these points into consideration together with the fact of small sample size , no solid conclusion could be made from these results.

Determination of antimicrobial sensitivity of isolated bacteria to different antibiotics was another aim of this study. Eleven (11) different antibiotics disks with
predetermined concentrations from different groups were studied. Their effect on bacterial growth was identified by Kirby – Bauer antibiotic disc diffusion method. The result were interpreted according to CLSI guidelines (2000) by measuring the diameter of area of inhibition in mm. The result of the current research indicated that all the Staphylococcus aureus isolates were 100% resistant to ampicillin, ceftriaxone and erythromycin. The results also showed high resistance rate to cefotaxime (67%). Staphylococcal resistance to penicillins and cephalosporins is an expected finding since all the Staphylococcal aureus microorganisms are able to produce penicillinase or (beta-lactamase) enzyme which rapidly inactivates penicillin (or cephalosporin) by breaking down beta-lactam ring(35). In addition, other mechanisms may also be contributed to the resistance of Staphylococcus aureus to penicillins and cephalosporins such as expression of Penicillin Binding Protein-2a (PBP-2a)(36) and PBP-4(37). Staphylococcal aureus resistance to erythromycin (and macrolides) is reported in our country 38,39 and in nearby countries like Jordan(40) and Iran(41). The resistance rate varied from as low as 25%(38), 41%(40), 53%(41) and up to 55%(39). The result of the current study showed higher resistance rate to erythromycin than those reported in literature. Possible explanations include small sample size used in this study, frequency of usage of the individual antibiotics in hospitals compared to outpatients, or probably real differences in resistance rate between children and adults. Meanwhile, all the Staphylococcal aureus isolates were sensitive to meropenem (100%) followed by levofloxacin and amoxiclav (88% and 87% respectively). Sensitivity of Staphylococcus aureus to above mentioned antibiotics varies greatly in literature. In Iran, Sultani et al (2012)(42) reported very low sensitivity rate (1%) of Staphylococcus aureus to meropenem and ciprofloxacin from different nosocomial infections. Whereas in India, Batabyal et al (2012)(43) demonstrated 35% sensitivity rate to meropenem and 17.6 % to ciprofloxacin and 11.8% to amoxiclav in postoperative oral and fascio-maxillary infections. On other hand, Brethis et al (2019)(44) in India, has found a much higher sensitivity rate of Staphylococcus aureus to meropenem (76%), ciprofloxacin (61%) and amoxiclav (34%). Moreover, Burki et al (2014)(45) in Pakistan detected 100 % and 93% sensitivity rate of meticillin resistant Staphylococcus aureus (MRSA) to levofloxacin and meropenem respectively in intra-abdominal infections. Taking into considerations these variable results, one can consider possible contributing reasons including discrimination between MRSA and non MRSA, different geographic locations, different infection sites, different age distributions and different sample size. These possible contributing factors are supported in literature by surveillance trials(46).

Regarding the antibiogram profile of Esch.coli, the result of the current study demonstrated 100% resistance rate to several antibiotics such as ampicillin, cefotaxime, ceftriaxone, erythromycin and nalidixic acid. Multidrug resistant (MDR) Esch.coli is now a global concern with increasing resistant rate worldwide(47,48). In Iraq and many other countries, several studies showed increasing frequency of MDR Esch. coli in different infections like UTI(49,50,51,52,53), bacteremia(49) and children diarrhea(54). These studies showed high resistance rate to a wide range of antibiotics including ampicillin, cephalosporin and nalidixic acid. Increasing resistance rate of E.coli to different antibiotics might be attributed to the emergence of extended spectrum beta-lactamase (ESBL) producing E.coli due to the fact that beta-lactam antibiotics are commonly used to treat suspected Esch.coli infections like UTI and diarrhea(55). Meanwhile, the irrational use of penicillins and third generation cephalosporins, mainly ceftriaxone and cefotaxime in the hospitals can be an important contributing factor for increasing resistance rate(50). Furthermore, the lack
of antibiotic policy, self-medication practice, ease of getting antibiotics from market without doctor’s prescription in third world countries all considered important additional factors for antimicrobial resistance Esch. coli. Although the results of the current study showed higher resistance rate of Esch.coli to different antibiotics than above studies, it raises the alarm of increasing frequency of this problem in our country. On other hand, high sensitivity rate were reported with amoxiclav, norfloxacin, levofloxacin, meropenem and chloramphenicol. Apart from amoxiclav, high sensitivity rate to above antibiotics were reported in other studies in Iraq. In this context, Al-Aaboda and Al-Notazy (2018)(51) demonstrated high sensitivity rate of Esch.coli to norfloxacin and chloramphenicol in patients with UTI in adults. Similarly, Mohammed et al (2014)(49) showed high sensitivity rate of Esch.coli isolates to chloramphenicol (81%) and imipenem (100%). In addition, Polse et al (2016)(56) also demonstrated 100 % sensitivity rate to imipenem and meropenem. These drugs resist the effect of beta lactamases enzymes produced by extended spectrum beta lactamase ESBL producing bacteria, making them most effective drugs used in the treatment of multi-drug resistant MDR Esch.coli(57) However, their only intravenous or intramuscular route of administration limit their use by most doctors(57)

Regarding Pseudomonas aeruginosa, the result of the present study showed 100 % resistant rate to ampicillin, cephalosporins, amoxiclav, erythromycin, chloramphenicol and azithromycin. These findings are not surprising since Pseudomonas aeruginosa are known to be resistant to these antibiotics due to various combinations of intrinsic and extrinsic mechanisms including de-repression of chromosomal beta-lactamase, overexpression of the MexAB-OprM multidrug efflux pump due to a Nal B mutation, impermeability type resistance, plasmid mediated production of modifying enzymes and target site mutations in the GyrA(58). Moreover, high resistance rate was also reported with Meropenem (62%). In these context, loss of / reduction in levels of specific outer membrane porin channel, OprD, due to an NfxC mutation and overexpression of the MexAB-OprM multidrug efflux pump due to a NalB mutation are suggested mechanisms for increasing resistance rate of Pseudomonas aeruginosa to above mentioned antibiotic(58). These results are comparable with the results of some other investigators in Iraq such as Shilba et al (2015)(59) and AL-Rubaye et al (2015)(60) who found 67% and 77 % resistance rate of Pseudomonas aeruginosa to meropenem respectively. However, they are higher than those reported by Hussein et al (2018)(61) who reported only here 35% resistance rate to meropenem.

**Conclusion**

*Staphylococcus aureus* in the blood, and *Staphylococcus aureus* and *Escherichia coli* from the urine, remain the most common bacterial isolates in infants less than one month of age with severe bacterial infections. Both of them are highly sensitive to amoxiclav, levofloxacin and meropenem and highly resistant to ampicillins and 3rd generation cephalosporins. *Pseudomonas aeruginosa* are only moderately sensitive to meropenem and are resistant to most commonly used antibiotics. While meropenem (and carbencillin in general) is considered effective against most of isolated bacteria, their use should be highly restricted to most serious infections to avoid the possibility of dissemination of carbencillin resistant infections.
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